

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number
WO 03/022208 A2

- (51) International Patent Classification⁷: **A61K**
- (74) Agent: **MYERS BIGEL SIBLEY & SAJOVEC**; PO Box 37428, Raleigh, NC 27627 (US).
- (21) International Application Number: **PCT/US02/28429**
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date:
6 September 2002 (06.09.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/318,193 7 September 2001 (07.09.2001) US
60/377,865 3 May 2002 (03.05.2002) US
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): **NOBEX CORPORATION** [US/US]; Post Office Box 13940, Research Triangle Park, NC 27709-3940 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **SOLTERO, Richard** [US/US]; 5509 Solomon's Seal Court, Holly Springs, NC 27540 (US). **RADHAKRISHNAN, Balasingham** [US/US]; 209 Faison Road, Chapel Hill, NC 27517 (US). **EKWURIBE, Nnochiri, N.** [NG/US]; 216 Coltsgate Dr., Cary, NC 27511 (US). **REHLAENDER, Bruce** [US/US]; 604 Highgrove Drive, Chapel Hill, NC 27516 (US). **HICKEY, Anthony** [GB/US]; 1208 Killington Court, Chapel Hill, NC 27517 (US). **BOVET, Li, Li** [US/US]; 110 Marin Drive, Chapel Hill, NC 27516 (US).
- Published:**
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 03/022208 A2

(54) Title: **PHARMACEUTICAL COMPOSITIONS OF INSULIN DRUG-OLIGOMER CONJUGATES AND METHODS OF TREATING DISEASES THEREWITH**

(57) Abstract: Pharmaceutical compositions that include an insulin drug-oligomer conjugate, a fatty acid component, and a bile salt component are described. The insulin drug is covalently coupled to an oligomeric moiety. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. Methods of treating an insulin deficiency in a subject in need of such treatment using such pharmaceutical compositions are also provided, as are methods of providing such pharmaceutical compositions.

**PHARMACEUTICAL COMPOSITIONS OF INSULIN DRUG-OLIGOMER
CONJUGATES AND METHODS OF TREATING DISEASES THEREWITH**

Related Application

This application claims the benefit of United States Provisional Application Number 60/318,193, filed September 7, 2001 and United States Provisional Application Number 60/377,865, filed May 3, 2002, the disclosures of which are incorporated herein by reference in their entireties.

5

Field of the Invention

The present invention relates to pharmaceutical compositions and methods of treating diseases therewith.

10

Background of the Invention

The polypeptide insulin is the primary hormone responsible for controlling the transport, utilization and storage of glucose in the body. The β -cells of the pancreatic islets secrete a single chain precursor of insulin, known as proinsulin. Proteolysis of proinsulin results in removal of certain basic amino acids in the proinsulin chain and the connecting or C-peptide and provides the biologically active polypeptide insulin.

15

The insulin molecule has been highly conserved in evolution and generally consists of two chains of amino acids linked by disulfide bonds. In the natural human, two-chain insulin molecule (mw 5,800 Daltons), the A-chain is composed of 21 amino acid residues and has glycine at the amino terminus; and the B-chain has 30 amino acid residues and phenylalanine at the amino terminus.

20

Insulin may exist as a monomer or may aggregate into a dimer or a hexamer formed from three of the dimers. Biological activity, i.e., the ability to bind to receptors and stimulate the biological actions of insulin, resides in the monomer.

Diabetes is a biological disorder involving improper carbohydrate metabolism.

5 Diabetes results from insufficient production of or reduced sensitivity to insulin. In persons with diabetes, the normal ability to use glucose is inhibited, thereby increasing blood sugar levels (hyperglycemia). As glucose accumulates in the blood, excess levels of sugar are excreted in the urine (glycosuria). Other symptoms of diabetes include increased urinary volume and frequency, thirst, itching, hunger, weight loss, and weakness.

10 There are two varieties of diabetes. Type I is insulin-dependent diabetes mellitus, or IDDM. IDDM was formerly referred to as "juvenile onset diabetes." In IDDM, insulin is not secreted by the pancreas and must be provided from an external source. Type II or adult-onset diabetes can ordinarily be controlled by diet, although in some advanced cases insulin is required.

15 Before the isolation of insulin in the 1920s, most patients died within a short time after onset. Untreated diabetes leads to ketosis, the accumulation of ketones, products of fat breakdown, in the blood. This is followed by the accumulation of acid in the blood (acidosis) with nausea and vomiting. As the toxic products of disordered carbohydrate and fat metabolism continue to build up, the patient goes into a diabetic coma, which leads to death.

20 The use of insulin as a treatment for diabetes dates to 1922, when Banting et al. ("Pancreatic Extracts in the Treatment of Diabetes Mellitus," Can. Med. Assoc. J., 12:141-146 (1922)) showed that the active extract from the pancreas had therapeutic effects in diabetic dogs. In that same year, treatment of a diabetic patient with pancreatic extracts resulted in a dramatic, life-saving clinical improvement.

25 Until recently, bovine and porcine insulin were used almost exclusively to treat diabetes in humans. Today, however, numerous variations in insulin between species are known. Each variation differs from natural human insulin in having amino acid substitution(s) at one or more positions in the A- and/or B-chain. Despite these differences, most mammalian insulin has comparable biological activity. The advent of recombinant
30 technology allows commercial scale manufacture of human insulin (e.g., Humulin™ insulin, commercially available from Eli Lilly and Company, Indianapolis, IN) or genetically engineered insulin having biological activity comparable to natural human insulin.

Treatment of diabetes typically requires regular injections of insulin. Due to the inconvenience of insulin injections, various approaches have been attempted to formulate insulin for administration by non-injectable routes.

For example, U.S. Patent No. 4,338,306 to Kitao et al. proposes pharmaceutical compositions for rectal administration of insulin. The pharmaceutical compositions include insulin and fatty acids having 8 to 14 carbon atoms and nontoxic salts thereof.

U.S. Patent No. 4,579,730 to Kidron et al. proposes pharmaceutical compositions for the oral administration of insulin. The pharmaceutical compositions include insulin, a bile acid or alkali metal salt thereof, the bile acid being selected from the group consisting of cholic acid, chenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, glycocholic acid, glycochenocholic acid, 3 β -hydroxy-12-ketocholic acid, 12 α -3 β -dihydrocholic acid, and ursodesoxycholic acid, and a protease inhibitor. The composition is provided with an enterocoating to assure passage through the stomach and release in the intestine.

U.S. Patent No. 5,283,236 to Chiou proposes compositions for systemic delivery of insulin through the eyes where the drug passes into the nasolacrimal duct and becomes absorbed into circulation. The composition includes insulin and an enhancing agent. The enhancing agents proposed include, either alone or in combination, surfactants such as polyoxyethylene ethers of fatty acids and bile salts and acids such as cholic acid, deoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium cholate, sodium glycocholate, glycocholate, sodium deoxycholate, sodium taurodeoxycholate, chenodeoxycholic acid, and ursodeoxycholic acid. The enhancer is present in a concentration ranging from 0.1% to 5% (w/v).

U.S. Patent No. 5,658,878 to Bäckström et al. proposes a therapeutic preparation for inhalation that includes insulin and a substance which enhances the absorption of insulin in the lower respiratory tract. The enhancer is preferably a sodium salt of a saturated fatty acid of carbon chain length 10 (i.e., sodium caprate), 12 (sodium laurate), or 14 (sodium myristate). Potassium and lysine salts of capric acid are also proposed. Bäckström et al. note that if the carbon chain length is shorter than about 10, the surface activity of the surfactant may be too low, and if the chain length is longer than about 14, decreased solubility of the fatty acid in water limits its usefulness. As an alternative to the proposed fatty acid enhancers, Bäckström et al. propose the use of the following bile salts - sodium ursodeoxycholate, sodium taurocholate, sodium glycocholate, and sodium taurodihydrofusidate.

U.S. Patent No. 5,853,748 to New proposes enteric-coated compositions for oral administration of insulin. The composition includes insulin, a bile salt or bile acid, and carbonate or bicarbonate ions, which are used to adjust the pH of the gut to a pH of from 7.5 to 9.

5 U.S. Patent No. 6,200,602 to Watts et al. proposes drug delivery compositions for colonic delivery of insulin. The drug delivery compositions include insulin, an absorption promoter which (a) includes a mixture of fatty acids having 6 to 16 carbon atoms or a salt thereof and a dispersing agent, or (b) comprises a mixture of mono/diglycerides of medium chain fatty acids and a dispersing agent, and a coating to prevent the release of the insulin and
10 absorption promoter until the tablet, capsule or pellet reaches the proximal colon.

It is desirable to provide pharmaceutical compositions for administration of insulin that may provide improved bioavailability when compared to the conventional compositions described above.

15

Summary of the Invention

Pharmaceutical compositions according to embodiments of the present invention use a mixture of bile salts and fatty acids in a particular ratio that appears to provide synergistic effects in the administration of insulin drug-oligomer conjugates that may not be achieved with bile salts or fatty acids alone. For example, in some embodiments of the present
20 invention, using mixtures of bile salts and fatty acids in a particular ratio alters the precipitation characteristics of the bile salt so that the bile salt more readily re-solubilizes if it happens to precipitate out of the pharmaceutical composition (e.g, upon encountering an acidic environment in the gut). As another example, in some embodiments of the present invention, using mixtures of bile salts and fatty acids in a particular ratio lowers the
25 precipitation point of the bile salt in the pharmaceutical composition, providing additional buffering capacity for the pharmaceutical composition.

According to embodiments of the present invention, a pharmaceutical composition includes an insulin drug-oligomer conjugate that includes an insulin drug covalently coupled to an oligomeric moiety, a fatty acid component that includes a fatty acid, and a bile salt
30 component that includes a bile salt. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition. The bile salt component is present in an amount sufficient to

lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition.

According to other embodiments of the present invention, a pharmaceutical composition includes an insulin drug-oligomer conjugate that includes an insulin drug covalently coupled to an oligomeric moiety, a bile salt component comprising a bile salt, and a fatty acid component comprising a fatty acid. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component is present in a first amount such that, at the precipitation point of the bile salt, the bile salt precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were not present in the composition.

According to still other embodiments of the present invention, a pharmaceutical composition includes an insulin drug-oligomer conjugate that includes an insulin drug covalently coupled to an oligomeric moiety, between 0.1 and 15% (w/v) of a fatty acid component, and between 0.1 and 15% (w/v) of a bile salt component. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1.

According to other embodiments of the present invention, methods of treating an insulin deficiency in a subject in need of such treatment include administering to the subject a pharmaceutical composition according to embodiments of the present invention.

According to still other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a bile salt to include in the composition based on the ability of the bile salt to increase the solubility of a fatty acid component when the composition has a pH of 8.5 or less.

According to yet other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to lower the precipitation point of a bile salt component in the composition to a pH of 5.5 or less.

According to other embodiments of the present invention, a method of providing a pharmaceutical composition includes selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to alter the precipitation characteristics of a bile salt component in the composition.

Brief Description of the Drawings

Figure 1 illustrates a comparison of mean plasma glucose vs. time curves resulting from oral administration of various doses of embodiments of the present invention in fasting, non-diabetic subjects compared with a mean plasma glucose vs. time curve for baseline plasma glucose;

Figure 2 illustrates a comparison of mean plasma insulin vs. time curve resulting from oral administration of various doses of embodiments of the present invention in fasting, non-diabetic subjects compared with a mean plasma insulin vs. time curve for baseline plasma insulin;

Figure 3 illustrates glucose and insulin dose responses resulting from oral administration of embodiments of the present invention in fasting, non-diabetic subjects;

Figure 4 illustrates a comparison of mean plasma glucose vs. time curves resulting from post-prandial, oral administration of various doses of embodiments of the present invention in non-diabetic subjects compared with a mean plasma glucose vs. time curve for baseline plasma glucose;

Figure 5 illustrates a comparison of mean plasma insulin vs. time curve resulting from post-prandial, oral administration of various doses of embodiments of the present invention in non-diabetic subjects compared with a mean plasma insulin vs. time curve for baseline plasma insulin;

Figure 6 illustrates glucose and insulin dose responses resulting from post-prandial oral administration of embodiments of the present invention in non-diabetic subjects;

Figure 7 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice;

Figure 8 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice; and

Figure 9 illustrates a blood glucose vs. time curve resulting from oral administration of embodiments of the present invention in mice.

Detailed Description of Preferred Embodiments

The present invention now will be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are

provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

All amino acid abbreviations used in this disclosure are those accepted by the United States Patent and Trademark Office as set forth in 37 C.F.R. § 1.822(b).

5 As used herein, the term "between" when used to describe various ranges should be interpreted to include the end-points of the described ranges.

As used herein, the term "substantially monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight.

10 As used herein, the term "monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "insulin polypeptide" means a polypeptide possessing at least some of the biological activity of insulin (e.g., ability to affect the body through insulin's primary mechanism of action). For example, an insulin polypeptide may be a polypeptide
15 such as insulin having an A-chain polypeptide and a B-chain polypeptide coupled to the A-chain polypeptide by disulfide bonds. In various embodiments of the present invention, the insulin polypeptide preferably possesses a majority of the biological activity of insulin, more preferably possesses substantially all of the biological activity of insulin, and most preferably possesses all of the biological activity of insulin.

20 As used herein, the term "insulin" means the insulin of one of the following species human, cow, pig, sheep, horse, dog, chicken, duck, whale, or the like provided by natural, synthetic, or genetically engineered sources. In various embodiments of the present invention, insulin is preferably human insulin.

As used herein, the term "insulin analog" means insulin wherein one or more of the
25 amino acids have been replaced while retaining some or all of the activity of the insulin. The analog is described by noting the replacement amino acids with the position of the replacement as a superscript followed by a description of the insulin. For example, "Pro^{B29} insulin, human" means that the lysine typically found at the B29 position of a human insulin molecule has been replaced with proline.

30 Insulin analogs may be obtained by various means, as will be understood by those skilled in the art. For example, certain amino acids may be substituted for other amino acids in the insulin structure without eliminating a therapeutically beneficial effect. As the interactive capacity and nature of insulin defines its biological functional activity, certain amino acid sequence substitutions can be made in the amino acid sequence and nevertheless

remain a polypeptide without eliminating a therapeutically beneficial effect.

In making such substitutions, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). As will be understood by those skilled in the art, certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity, *i.e.*, still obtain a biological functionally equivalent polypeptide. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 of each other is preferred, those which are within ± 1 of each other are particularly preferred, and those within ± 0.5 of each other are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, the disclosure of which is incorporated herein in its entirety, provides that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (± 3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). As is understood by those skilled in the art, an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 of each other is preferred, those which are within ± 1 of each other are particularly preferred, and those within ± 0.5 of each other are even more particularly

preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions (i.e., amino acids that may be interchanged without significantly altering the biological activity of the polypeptide) that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include, for example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

As will be understood by those skilled in the art, insulin analogs may be prepared by a variety of recognized peptide synthesis techniques including, but not limited to, classical (solution) methods, solid phase methods, semi-synthetic methods, and recombinant DNA methods.

Examples of human insulin analogs include, but are not limited to, Gly^{A21} insulin, human; Gly^{A21} Gln^{B3} insulin, human; Ala^{A21} insulin, human; Ala^{A21} Gln^{B3} insulin, human; Gln^{B3} insulin, human; Gln^{B30} insulin, human; Gly^{A21} Glu^{B30} insulin, human; Gly^{A21} Gln^{B3} Glu^{B30} insulin, human; Gln^{B3} Glu^{B30} insulin, human; Asp^{B28} insulin, human; Lys^{B28} insulin, human; Leu^{B28} insulin, human; Val^{B28} insulin, human; Ala^{B28} insulin, human; Asp^{B28} Pro^{B29} insulin, human; Lys^{B28} Pro^{B29} insulin, human; Leu^{B28} Pro^{B29} insulin, human; Val^{B28} Pro^{B29} insulin, human; Ala^{B28} Pro^{B29} insulin, human.

As used herein, the term "insulin fragment" means a segment of the amino acid sequence found in the insulin that retains some or all of the activity of the insulin. Insulin fragments are denoted by stating the position(s) in an amino acid sequence followed by a description of the amino acid. For example, a "B25-B30 human insulin" fragment would be the six amino acid sequence corresponding to the B25, B26, B27, B28, B29 and B30 positions in the human insulin amino acid sequence.

As used herein, the term "insulin fragment analog" means a segment of the amino acid sequence found in the insulin molecule wherein one or more of the amino acids in the segment have been replaced while retaining some or all of the activity of the insulin.

As used herein, the term "polypeptide" means a peptide having two or more amino acid residues.

As used herein, the term "amphiphilically balanced" means capable of substantially dissolving in water and capable of penetrating biological membranes.

As used herein, the term "polyalkylene glycol" refers to straight or branched polyalkylene glycol polymers such as polyethylene glycol, polypropylene glycol, and

polybutylene glycol, and includes the monoalkylether of the polyalkylene glycol. The term "polyalkylene glycol subunit" refers to a single polyalkylene glycol unit. For example, a polyethylene glycol subunit would be $-O-CH_2-CH_2-O-$.

As used herein, the term "lipophilic" means the ability to dissolve in lipids and/or the ability to penetrate, interact with and/or traverse biological membranes, and the term, "lipophilic moiety" or "lipophile" means a moiety which is lipophilic and/or which, when attached to another chemical entity, increases the lipophilicity of such chemical entity. Examples of lipophilic moieties include, but are not limited to, alkyls, fatty acids, esters of fatty acids, cholesteryl, adamantyl and the like.

As used herein, the term "lower alkyl" refers to substituted or unsubstituted alkyl moieties having from one to five carbon atoms.

As used herein, the term "higher alkyl" refers to substituted or unsubstituted alkyl moieties having six or more carbon atoms.

Unless otherwise noted herein, the term "bile salt" includes bile salts and the free acids thereof.

Unless otherwise noted herein, the term "fatty acid" includes fatty acids and pharmaceutically acceptable salts or esters thereof.

As used herein, the term "bile salt component" means a mixture of one or more salts.

As used herein, the term "fatty acid component" means a mixture of one or more fatty acids.

As used herein, the "precipitation point" of a compound or component of the pharmaceutical composition is the pH at which at least 25% of the compound or component precipitates out of the composition. Accordingly, lowering the precipitation point means lowering the pH at which at least 25% of the compound or component precipitates out of the composition. Conversely, raising the precipitation point means raising the pH at which at least 25% of the compound or component precipitates out of the composition.

As used herein, the "solubility point" of a compound or component of the pharmaceutical composition is the pH at which at least 75% of the compound or component is solubilized in the composition. Accordingly, lowering the solubility point means lowering the pH at which at least 75% of the compound or component is solubilized in the composition. Conversely, raising the solubility point means raising the pH at which at least 75% of the compound or component is solubilized in the composition.

As used herein, the term "medium-chain fatty acid" means a saturated or unsaturated fatty acid having from 8 to 14 carbon atoms.

As used herein, the term "long-chain fatty acid" means a saturated or unsaturated fatty acid having greater than 14 carbon atoms.

According to embodiments of the present invention, a pharmaceutical composition comprises an insulin drug-oligomer conjugate, a fatty acid component, and a bile salt component. The insulin drug-oligomer conjugate includes an insulin drug covalently coupled to an oligomeric moiety. The fatty acid component includes a fatty acid, and the bile salt component includes a bile salt.

According to these embodiments of the present invention, the fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. The fatty acid component and the bile salt component are preferably present in a weight-to-weight ratio of between 1:3 and 3:1 and more preferably present in a weight-to-weight ratio of between 1:2 and 2:1.

According to some embodiments of the present invention, the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition. The fatty acid component is preferably present in an amount sufficient to lower the precipitation point of the bile salt by at least 0.5 pH units, and is more preferably present in an amount sufficient to lower the precipitation point of the bile salt by at least 1.0 pH units.

According to other embodiments of the present invention, the bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition. The bile salt component is preferably present in an amount sufficient to lower the solubility point of the fatty acid by at least 0.25 pH units, and is more preferably present in an amount sufficient to lower the solubility point of the fatty acid by at least 0.5 pH units.

According to still other embodiments of the present invention, the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid were not present in the pharmaceutical composition as described above, and the bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition as described above.

The bile salt in the bile salt component may be various bile salts as will be understood by those skilled in the art including unconjugated and conjugated bile salts. Unconjugated

bile salts are bile salts in which the primary side chain has a single carboxyl group which is at the terminal position and which is unsubstituted. Exemplary unconjugated bile salts include, but are not limited to, cholate, ursodeoxycholate, chenodeoxycholate, and deoxycholate.

Conjugated bile salts are bile salts in which the primary side chain has a carboxyl group

5 which is substituted with, for example, an amino acid derivative linked via its nitrogen atom to the carboxyl group. Exemplary conjugated bile salts include, but are not limited to, taurocholate, glycocholate, taurodeoxycholate, and glycodeoxycholate. Mixtures of the various unconjugated and/or conjugated bile salts may also be used. The bile salt is preferably a pharmaceutically acceptable salt of cholic acid. More preferably, the bile salt is
10 sodium cholate. Still more preferably, the bile salt component consists essentially of sodium cholate.

The fatty acid in the fatty acid component may be various fatty acids as will be understood by those skilled in the art including natural and synthetic fatty acids. The fatty acid preferably has between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
15 17, 18, or 19 carbon atoms and an upper limit of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 carbon atoms. The fatty acid may be either saturated or unsaturated. Exemplary saturated fatty acids include, but are not limited to, ethanoic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, tridecanoic acid, tetradecanoic acid,
20 pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, nonadecanoic acid, eicosanoic acid, one or more of which may be referred to by their common names such as acetic acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, and cerotic acid. Exemplary unsaturated fatty acids include, but are not limited to, cis-9-octadecenoic acid, trans-9-
25 octadecenoic acid, 9,12-octadecatrienoic acid, 9,12,15-octadecenoic acid, and 5,8,11,14-eicosatetraenoic acid, one or more of which may be referred to by their common names such as oleic acid, elaidic acid, linoleic acid, linolenic acid, and arachidonic acid.

In some embodiments, the fatty acid component comprises a mixture of two or more fatty acids. In other embodiments, the fatty acid component comprises a medium-chain fatty
30 acid and a long-chain fatty acid. The medium-chain fatty acid is preferably capric acid, lauric acid, or a mixture thereof. The long-chain fatty acid is preferably oleic acid.

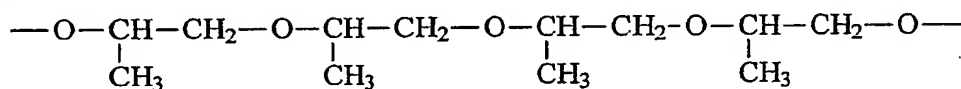
The insulin drug may be various insulin drugs as will be understood by those skilled in the art. The insulin drug is preferably an insulin polypeptide. The insulin polypeptide preferably has an A-chain polypeptide and a B-chain polypeptide. The A-chain polypeptide

is preferably devoid of lysine residues. The B-chain polypeptide preferably comprises a single lysine residue. The A-chain polypeptide and the B-chain polypeptide are preferably cross-linked, and are more preferably cross-linked using one or more disulfide bonds. Still more preferably, the A-chain polypeptide and the B-chain polypeptide each comprise
5 cysteine residues, one or more of which are coupled using one or more disulfide bonds to cross-link the A-chain polypeptide with the B-chain polypeptide. Preferably, the insulin polypeptide is insulin, an insulin analog, an insulin fragment, or an insulin analog fragment. More preferably, the insulin polypeptide is human insulin, a human insulin analog, a human insulin fragment, or a human insulin analog fragment.

10 The oligomer may be various oligomers as will be understood by those skilled in the art. In general, the oligomer may be any oligomer capable of being coupled to a polypeptide as will be understood by those skilled in the art. For example, the oligomer may be a poly-dispersed oligomer as described in U.S. Patent No. 4,179,337 to Davis et al.; U.S. Patent No. 5,567,422 to Greenwald; U.S. Patent No. 5,359,030 to Ekwuribe; U.S. Patent No. 5,438,040
15 to Ekwuribe, U.S. Patent No. 5,681,811 to Ekwuribe, and U.S. Patent No. 6,309,633 to Ekwuribe et al., the disclosures of each of which are incorporated herein by reference in their entireties. As another example, the oligomer may be a non-polydispersed oligomer as described in U.S. Patent Application Serial No. 09/873,731 filed June 4, 2001 by Ekwuribe et al. entitled "Methods of Synthesizing Substantially Monodispersed Mixtures of Polymers
20 Having Polyethylene Glycol Mixtures"; U.S. Patent Application Serial No. 09/873,797 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; and U.S. Patent Application Serial No. 09/873,899 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Insulin Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and
25 Methods of Making Same," the disclosures of each of which are incorporated herein in their entireties.

In some embodiments, the oligomer comprises a hydrophilic moiety as will be understood by those skilled in the art including, but not limited to, polyalkylene glycols such as polyethylene glycol or polypropylene glycol, polyoxyethylenated polyols, copolymers
30 thereof and block copolymers thereof, provided that the hydrophilicity of the block copolymers is maintained. The hydrophilic moiety is preferably a polyalkylene glycol moiety. The polyalkylene glycol moiety has at least 1, 2, 3, 4, 5, 6 or 7 polyalkylene glycol subunits. The polyalkylene glycol moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,

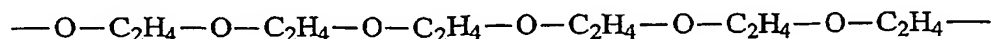
31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49 polyalkylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more polyalkylene glycol subunits. The polyalkylene glycol moiety
 5 more preferably has between a lower limit of 2, 3, 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 polyalkylene glycol subunits. Even more preferably, the polyalkylene glycol moiety has between a lower limit of 3, 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits. The polyalkylene glycol moiety still more preferably has
 10 between a lower limit of 4, 5, or 6 polyalkylene glycol subunits and an upper limit of 6, 7, or 8 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene glycol moiety is a polypropylene glycol moiety, the moiety preferably has a uniform (i.e., not
 15 random) structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



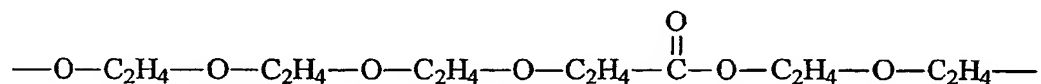
This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such
 20 uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, additional hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in
 25 the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more additional hydrophilic moieties (i.e., moieties in addition to the polyalkylene glycol moiety) including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol
 30 moieties will be considered to be the same moiety if they are coupled by ether bonds. For example, the moiety



is a single polyethylene glycol moiety having six polyethylene glycol subunits. If this moiety were the only hydrophilic moiety in the oligomer, the oligomer would not contain an additional hydrophilic moiety. Adjacent polyethylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond. For example,
 5 the moiety



is a polyethylene glycol moiety having four polyethylene glycol subunits and an additional hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety
 10 and no additional hydrophilic moieties.

The oligomer preferably further comprises one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety has at least 1, 2, 3, 4, 5, or 6 carbon atoms. The lipophilic moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 carbon
 15 atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms. The lipophilic moiety more preferably has between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 carbon atoms. The lipophilic moiety even more preferably has between a lower limit of 3, 4, 5, 6, 7, 8, or 9 carbon atoms
 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 carbon atoms. The lipophilic moiety still more preferably has between a lower limit of 3, 4, 5, 6, or 7 carbon atoms and an upper limit of 6, 7, 8, 9, or 10 carbon atoms. The lipophilic moiety is preferably selected from the group consisting of saturated or unsaturated, linear or branched alkyl moieties, saturated or unsaturated, linear or branched fatty acid moieties, cholesterol, and adamantane.

25 Exemplary alkyl moieties include, but are not limited to, saturated, linear alkyl moieties such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl and eicosyl; saturated, branched alkyl moieties such as isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl; and unsaturated alkyl moieties
 30 derived from the above saturated alkyl moieties including, but not limited to, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. Exemplary fatty acid moieties include, but are not limited to, unsaturated fatty acid moieties such as lauroleate,

myristoleate, palmitoleate, oleate, elaidate, erucate, linoleate, linolenate, arachidonate, eicosapentaenoate, and docosahexaenoate; and saturated fatty acid moieties such as acetate, caproate, caprylate, caprate, laurate, arachidate, behenate, lignocerate, and cerotate. The fatty acid moiety can be natural or synthetic.

5 The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the insulin polypeptide, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group
10 consisting of sugar, cholesterol and glycerine moieties. Sugar moieties may be various sugar moieties as will be understood by those skilled in the art including, but not limited to, monosaccharide moieties and disaccharide moieties. Preferred monosaccharide moieties have between 4 and 6 carbon atoms.

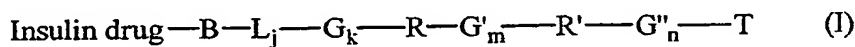
15 The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the insulin polypeptide as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties. The alkyl linker moiety may be a saturated or unsaturated, linear or branched alkyl moiety as will be understood by those skilled in the art including, but not limited to, methyl,
20 ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl, eicosyl, isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. The alkoxy moiety may be various alkoxy moieties including, but not limited to, methoxy,
25 ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, pentadecyloxy, hexadecyloxy, octadecyloxy, nonadecyloxy, eicosyloxy, isopropoxy, *sec*-butoxy, *tert*-butoxy, 2-methylbutoxy, *tert*-pentyloxy, 2-methyl-pentyloxy, 3-methylpentyloxy, 2-ethylhexyloxy, 2-propylpentyloxy, vinyloxy, allyloxy, 1-butenyloxy, 2-butenyloxy, ethynyloxy, 1-propynyloxy, and 2-propynyloxy. The alkyl linker moiety may have between a lower limit of
30 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms, and preferably has between 1, 2, 3, 4, or 5 carbon atoms and 8, 9, 10, 11, or 12 carbon atoms. The fatty acid linker moiety may be a saturated or

unsaturated, linear or branched fatty acid moiety as will be understood by those skilled in the art including, but not limited to, lauroleate, myristoleate, palmitoleate, oleate, elaidate, erucate, linoleate, linolenate, arachidonate, eicosapentaenoate, docosahexaenoate, acetate, caproate, caprylate, caprate, laurate, arachidate, behenate, lignocerate, and cerotate. The fatty acid linker moiety may be a natural or synthetic fatty acid. The fatty acid linker moiety may have between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms and preferably has between 1, 2, 3, 4, or 5 carbon atoms and 8, 10, 12, 14 or 16 carbon atoms. When the linker moiety is a fatty acid, the oligomeric moiety is preferably coupled to the insulin drug via the carbonyl group of a carboxylic acid moiety of the fatty acid.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer, which are not coupled to the insulin polypeptide. The terminating moiety is preferably an alkyl or alkoxy moiety. The alkyl or alkoxy moiety preferably has between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 carbon atoms. The alkyl or alkoxy moiety more preferably has between a lower limit of 1, 2, 3, 4, 5, 6, or 7 carbon atoms and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 carbon atoms. The alkyl or alkoxy moiety even more preferably has between a lower limit of 1, 2, 3, 4, or 5 carbon atoms and an upper limit of 5, 6, 7, 8, 9, or 10 carbon atoms. The alkyl or alkoxy moiety still more preferably has between a lower limit of 1, 2, 3, or 4 carbon atoms and an upper limit of 5, 6, or 7 carbon atoms. The alkyl moiety may be a linear or branched, saturated or unsaturated alkyl moiety as will be understood by those skilled in the art including, but not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, nonadecyl, eicosyl, isopropyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, *tert*-pentyl, 2-methyl-pentyl, 3-methylpentyl, 2-ethylhexyl, 2-propylpentyl, vinyl, allyl, 1-butenyl, 2-butenyl, ethynyl, 1-propynyl, and 2-propynyl. The alkoxy moiety may be various alkoxy moieties including, but not limited to, methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, pentadecyloxy, hexadecyloxy, octadecyloxy, nonadecyloxy, eicosyloxy, isopropoxy, *sec*-butoxy, *tert*-butoxy, 2-methylbutoxy, *tert*-pentyloxy, 2-methyl-pentyloxy, 3-methylpentyloxy, 2-ethylhexyloxy, 2-propylpentyloxy, vinyloxy, allyloxy, 1-butenyloxy, 2-butenyloxy, ethynyloxy, 1-propynyloxy, and 2-

propynyloxy. The terminating moiety is more preferably a lower alkyl moiety such as methyl, ethyl, propyl, isopropyl, butyl, *sec*-butyl, *tert*-butyl, pentyl, or *tert*-pentyl, or a lower alkoxy moiety such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, *sec*-butoxy, *tert*-butoxy, pentyloxy, or *tert*-pentyloxy. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

According to embodiments of the present invention, the insulin drug-oligomer conjugate comprises the structure of Formula I:

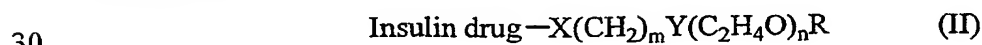


wherein:

- Insulin drug is a drug which is similar to those described above;
- B is a bonding moiety;
- L is a linker moiety;
- G, G' and G'' are individually selected spacer moieties;
- R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;
- T is a terminating moiety; and
- j, k, m and n are individually 0 or 1.

The bonding moiety is preferably selected from the group consisting of an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety, and a covalent bond. The linker moiety, spacer moieties, lipophilic moiety, polyalkylene glycol moiety, and terminating moiety are similar to those described above. Preferably, oligomers of these embodiments do not include spacer moieties (i.e., k, m and n are preferably 0).

In other embodiments, the insulin drug-oligomer conjugate comprises the structure of Formula II:



wherein:

- Insulin drug is a drug which is similar to those described above;
- X is -C(O)- or -O-;

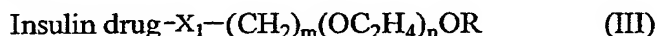
Y is an ester, an ether, a carbamate, a carbonate, or an amide bonding moiety, and is preferably an ether bonding moiety;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably has between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is more preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is even more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits, is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits, and is most preferably 7; and

R is a terminating moiety similar to those described above.

In still other embodiments, the insulin drug-oligomer conjugate comprises the structure of Formula III:



wherein:

Insulin drug is a drug which is similar to those described above;

X₁ is -C(O)- or -O-;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably has between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

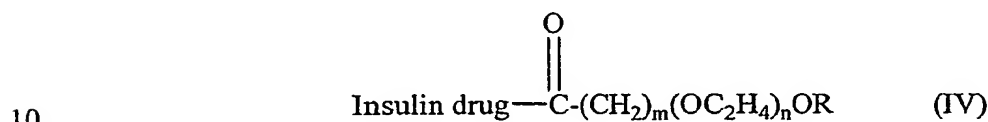
n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is more preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is even more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12

5 polyalkylene glycol subunits, is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits, and is most preferably 7; and

R is a terminating moiety similar to those described above.

In yet other embodiments, the insulin drug-oligomer conjugate comprises the structure of Formula IV:



wherein:

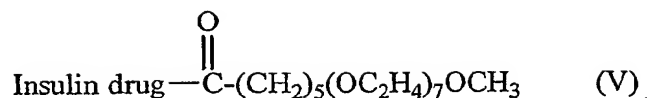
Insulin drug is a drug which is similar to those described above;

m is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10;

20 n is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, is more preferably between a lower limit of 2, 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, is even more preferably between a lower limit of 3, 4, 5, or 6 and an upper limit of 5, 6, 7, 8, 9, 10, 11, or 12 polyalkylene glycol subunits, and is still more preferably between a lower limit of 4, 5, or 6 and an upper limit of 6, 7, or 8 polyalkylene glycol subunits; and

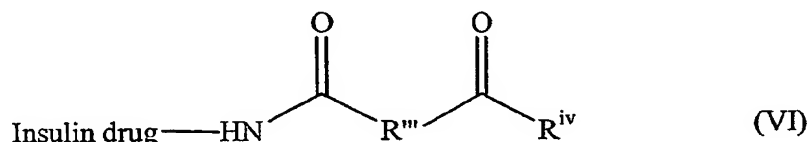
R is a terminating moiety similar to those described above.

30 In still other embodiments, the insulin drug-oligomer conjugate comprises the structure of Formula V:



wherein the insulin drug is a drug which is similar to those described above. When the insulin drug is a human insulin and the conjugate of Formula V consists of the single oligomer coupled to the Lysine at the B29 position of the human insulin, the insulin-oligomer conjugate is referred to as HIM2.

In still other embodiments of the present invention, the drug-oligomer conjugate comprises the structure of Formula VI:



wherein:

Insulin drug is a drug which is similar to those described above;

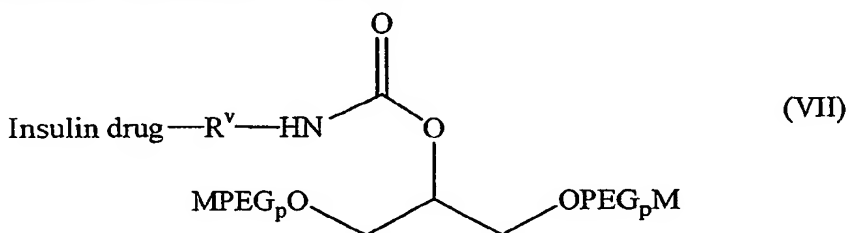
Rⁱⁱⁱ is a hydrophilic moiety; preferably a polyalkylene glycol moiety; more preferably a lower polyalkylene glycol moiety; and still more preferably a polyethylene glycol moiety or polypropylene glycol moiety, where the polyalkylene glycol moiety has at least 1, 2, or 3 polyalkylene glycol subunits; and R^{iv} is a lipophilic moiety; preferably an alkyl moiety having between 1 and 24 carbon atoms; more preferably a lower alkyl moiety; or

R^{iv} is a hydrophilic moiety; preferably a polyalkylene glycol moiety; more preferably a lower polyalkylene glycol moiety; and still more preferably a polyethylene glycol moiety or polypropylene glycol moiety, where the polyalkylene glycol moiety has at least 1, 2, or 3 polyalkylene glycol subunits; and Rⁱⁱⁱ is a lipophilic moiety; preferably an alkyl moiety having between 1 and 24 carbon atoms; more preferably a lower alkyl moiety; or

When the drug portion of the drug-oligomer conjugate of Formula VI is human insulin, Rⁱⁱⁱ is preferably polyethylene glycol having between a lower limit of 1, 2, 3, 4, 5, 6, or 7 polyethylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 polyethylene glycol subunits or Rⁱⁱⁱ is polypropylene glycol having between a lower limit of 1, 2, 3, 4, 5, 6, or 7 polypropylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 polypropylene glycol subunits, and R^{iv} is preferably alkyl having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. More preferably, Rⁱⁱⁱ is polyethylene glycol having between a lower limit of 1, 2, 3, or 4 polyethylene glycol subunits and an upper limit of 2, 3, 4, 5, 6, or 7 polyethylene glycol subunits or Rⁱⁱⁱ is polypropylene glycol having between a lower limit of 1, 2, 3, or 4 polypropylene glycol subunits and an

upper limit of 2, 3, 4, 5, 6, or 7 polypropylene glycol subunits, and R^{iv} is alkyl having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. Still more preferably, Rⁱⁱⁱ is polyethylene glycol having between a lower limit of 1, 2, or 3 polyethylene glycol subunits and an upper limit of 3, 4, or 5 polyethylene glycol subunits or Rⁱⁱⁱ is polypropylene glycol having between a lower limit of 1, 2, or 3 polypropylene glycol subunits and an upper limit of 3, 4, or 5 polypropylene glycol subunits, and R^{iv} is alkyl having 3, 4, 5, or 6 carbon atoms.

In yet other embodiments of the present invention, the insulin drug-oligomer conjugate comprises the structure of Formula VII:



10 wherein:

Insulin drug is a drug which is similar to those described above;

R^v is an alkyl or a fatty acid moiety as described above with reference to the lipophilic moiety; and

p is between a lower limit of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, is more preferably between a lower limit of 2, 3, 4, 5, 6, 7, 8, 9, or 10 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, is even more preferably between a lower limit of 3, 4, 5, 6, 7, 8, or 9 and an upper limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, and is still more preferably between a lower limit of 3, 4, 5, 6, or 7 and an upper limit of 6, 7, 8, 9, or 10.

In the various embodiments described above, the oligomer is covalently coupled to the insulin drug. In some embodiments, the oligomer is coupled to the insulin drug utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide an insulin drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the insulin drug-oligomer conjugate is biologically inactive (i.e., the conjugate lacks the ability to affect the body through the insulin polypeptide's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, providing the biologically active insulin drug over a given time period as one or more oligomers are cleaved from their respective biologically inactive insulin drug-oligomer conjugates to

provide the biologically active insulin drug. In other embodiments, the oligomer is coupled to the insulin drug utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the biologically inactive insulin drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

Oligomers employed in the various embodiments described above are commercially available or may be synthesized by various methods as will be understood by those skilled in the art. For example, polydispersed oligomers may be synthesized by the methods provided in one or more of the following references: U.S. Patent No. 4,179,337 to Davis et al.; U.S. Patent No. 5,567,422 to Greenwald; U.S. Patent No. 5,359,030 to Ekwuribe; U.S. Patent No. 5,438,040 to Ekwuribe, U.S. Patent No. 5,681,811 to Ekwuribe, U.S. Patent No. 6,309,633 to Ekwuribe et al. Non-polydispersed (e.g., substantially monodispersed and monodispersed) oligomers may be synthesized by methods provided in one or more of the following references: U.S. Patent Application Serial No. 09/873,731 filed June 4, 2001 by Ekwuribe et al. entitled "Methods of Synthesizing Substantially Monodispersed Mixtures of Polymers Having Polyethylene Glycol Mixtures"; U.S. Patent Application Serial No. 09/873,797 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same"; and U.S. Patent Application Serial No. 09/873,899 filed June 4, 2001 by Ekwuribe et al. entitled "Mixtures of Insulin Drug-Oligomer Conjugates Comprising Polyalkylene Glycol, Uses Thereof, and Methods of Making Same". Oligomers according to embodiments of the present invention are preferably substantially monodispersed and are more preferably monodispersed. Exemplary methods for synthesizing preferred monodispersed oligomers are provided in Examples 1 through 10 below.

In the various embodiments described above, more than one oligomer (i.e., a plurality of oligomers) may be coupled to the insulin drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the insulin drug, it may be preferable to couple one or more of the oligomers to the insulin drug with hydrolyzable bonds and couple one or more of the oligomers to the insulin drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the insulin drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the insulin drug by

hydrolysis in the body and one or more of the oligomers is slowly removed from the insulin drug by hydrolysis in the body.

The oligomer may be coupled to the insulin drug at various nucleophilic residues of the insulin drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the insulin drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the insulin polypeptide, the coupling preferably forms a secondary amine. When the insulin drug is human insulin, for example, the oligomer may be coupled to an amino functionality of the insulin, including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. Monoconjugates (i.e., when one oligomer is coupled to the insulin drug) are preferably prepared using methods described in U.S. Patent Application Serial No. 10/036,744, entitled "Methods of Synthesizing Insulin Polypeptide-Oligomer Conjugates, and Proinsulin Polypeptide-Oligomer Conjugates and Methods of Synthesizing Same," the disclosure of which is incorporated herein by reference in its entirety.

According to other embodiments of the present invention, a pharmaceutical composition comprises an insulin drug-oligomer conjugate, a fatty acid component, and a bile salt component. The insulin drug-oligomer conjugate includes an insulin drug covalently coupled to an oligomeric moiety. The fatty acid component includes a fatty acid, and the bile salt component includes a bile salt.

The insulin drug-oligomer conjugate includes an insulin drug covalently coupled to an oligomeric moiety, and is similar to the insulin drug-oligomer conjugates described above. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1, and are preferably present in a weight-to-weight ratio of between 1:3 and 3:1 and more preferably present in a weight-to-weight ratio of between 1:2 and 2:1. The fatty acid component is similar to the fatty acid components described above, and the bile salt component is similar to the bile salt components described above.

According to these embodiments of the present invention, the fatty acid component is present in a first amount such that, at the precipitation point of the bile salt, the bile salt precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were not present in the composition. The precipitation point of the bile salt in the formulation is preferably at or below a pH of 6.0, and is more preferably at or below a pH of 5.5. The pH above the precipitation point may be various pH's above the precipitation point. In some embodiments, the pH above the precipitation point is at least 0.5 pH units above the precipitation point. In other
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600
605
610
615
620
625
630
635
640
645
650
655
660
665
670
675
680
685
690
695
700
705
710
715
720
725
730
735
740
745
750
755
760
765
770
775
780
785
790
795
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995

In some embodiments, the first bile salt particles have an average diameter of less than 500 microns and the second bile salt particles have an average diameter of greater than 550 microns. In other embodiments, the first bile salt particles have an average diameter of less than 100 microns and the second bile salt particles have an average diameter of greater than 150 microns.

In some embodiments, the first bile salt particles are able to re-solubilize in less than 75% of the time it would have taken for the second bile salt particles to re-solubilize. In other embodiments, the first bile salt particles are able to re-solubilize in less than half the time it would have taken for the second bile salt particles to re-solubilize.

According to still other embodiments of the present invention, a pharmaceutical composition comprises an insulin drug-oligomer conjugate, between 0.1 and 15% (w/v) of a fatty acid component, and between 0.1 and 15% (w/v) of a bile salt component.

The insulin drug-oligomer conjugate includes an insulin drug covalently coupled to an oligomeric moiety, and is similar to the insulin drug-oligomer conjugates described above. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:3 and 3:1, and are preferably present in a weight-to-weight ratio of between 1:2 and 2:1. The fatty acid component is similar to the fatty acid components described above, and the bile salt component is similar to the bile salt components described above.

According to these embodiments of the present invention, the concentration of the fatty acid component is between a lower limit of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14% (w/v) and an upper limit of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 % (w/v). The concentration of the

fatty acid component is preferably between 0.5 and 10% (w/v), is more preferably between 0.5 and 5% (w/v), and is still more preferably between 1 and 3% (w/v).

According to these embodiments of the present invention, the concentration of the bile acid component is between a lower limit of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14% (w/v) and an upper limit of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 % (w/v). The concentration of the bile salt component is preferably between 0.5 and 10% (w/v), is more preferably between 1 and 5% (w/v), and is still more preferably between 2 and 4% (w/v).

Pharmaceutical compositions according to the present invention may further comprise a buffering component. The buffering component may comprise various buffering agents as will be understood by those skilled in the art. Exemplary buffering agents include, but are not limited to, inorganic acids (e.g., phosphoric acid), organic acids (e.g., citric acid), organic bases (e.g., tris-base (tris(hydroxymethyl)aminomethane), trolamine (triethanolamine), or histadine), and mixtures thereof. The buffering component preferably comprises an organic base, and more preferably comprises tris-base, trolamine, or a mixture thereof. In some embodiments, the buffering component comprises an organic acid and an organic base, and preferably comprises citric acid and tris-base, trolamine, or a mixture thereof. The buffering agent is preferably present in an amount that will buffer the pharmaceutical composition against the acidic environment that may be experienced in the gut as will be understood by one skilled in the art.

In addition to the bile salt component and fatty acid component, pharmaceutical compositions according to embodiments of the present invention may include various suitable excipients as will be understood by those skilled in the art, such as those found in the National Formulary 19, pages 2404-2406 (2000), the disclosure of pages 2404 to 2406 being incorporated herein in their entirety. For example, the pharmaceutical compositions may include lubricating agents such as, for example, talc, magnesium stearate and mineral oil; wetting agents; emulsifying and suspending agents; binding agents such as starches, gum arabic, microcrystalline cellulose, cellulose, methylcellulose, and syrup; anticaking agents such as calcium silicate; coating agents such as methacrylates and shellac; preserving agents such as methyl- and propyl hydroxybenzoates; sweetening agents; or flavoring agents. Polyols, and inert fillers may also be used. Examples of polyols include, but are not limited to, mannitol, sorbitol, xylitol, sucrose, maltose, glucose, lactose, dextrose, and the like. Other inert fillers which may be used encompass those which are known in the art and are useful in the manufacture of various dosage forms. If desired, the solid formulations may include

other components such as bulking agents and/or granulating agents, and the like. The drug products of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

5 The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, intracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal
10 administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular insulin drug-oligomer conjugate which is being used.

 Pharmaceutical compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined
15 amount of the insulin drug-oligomer conjugates; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the insulin drug-oligomer conjugate, the fatty acid component, the bile salt component, and a suitable carrier (which may contain one or
20 more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture.

25 In some embodiments of the present invention, the pharmaceutical composition is a liquid pharmaceutical composition suitable for oral administration. When the pharmaceutical composition is a liquid pharmaceutical composition, the composition preferably includes a buffering agent as described above. Liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is physiologically compatible.
30 Preferably, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 6.2 and 9.0. In some embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between a lower limit of 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7 and an upper limit of 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, or 8.9. In some

embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 7.0 and 8.5. In other embodiments, liquid pharmaceutical compositions according to embodiments of the present invention have a pH that is between 7.4 and 8.2.

5 In other embodiments of the present invention, the pharmaceutical composition is a solid pharmaceutical composition suitable for oral administration. The solid pharmaceutical composition may be prepared by various methods as will be understood by those skilled in the art. For example, a tablet may be prepared by compressing or molding a powder or granules containing the insulin drug-oligomer conjugate, the fatty acid component, the bile
10 salt component, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the mixture in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

15 Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component in a flavored base, usually an artificial sweetener and acacia or tragacanth; and pastilles comprising the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component in an inert base such as gelatin and glycerin or
20 sucrose and acacia.

Pharmaceutical compositions according to embodiments of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions comprising the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component, which preparations are preferably isotonic with the blood of the intended
25 recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit/dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring
30 only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition comprising the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component in a unit dosage form in a

sealed container may be provided. The mixture of the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the insulin drug-oligomer conjugate. When the insulin drug-oligomer conjugate is substantially water-insoluble, a sufficient amount of emulsifying agent which is physiologically acceptable may be employed in sufficient quantity to emulsify the insulin drug-oligomer conjugate in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Compositions suitable for transdermal administration may also be delivered by iontophoresis (*see, for example, Pharmaceutical Research* 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the insulin drug-oligomer conjugate, the fatty acid component, and the bile salt component. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

Methods of treating an insulin deficiency in a subject in need of such treatment by administering any of the various pharmaceutical compositions described above that contain a therapeutically effective amount of the insulin drug-oligomer conjugate are also provided. The effective amount of the insulin drug-oligomer conjugate, the use of which is in the scope of present invention, will vary somewhat from conjugate to conjugate, and subject to subject, and will depend upon factors such as the age and condition of the subject and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based

upon the weight of the insulin drug-oligomer conjugate. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base. A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of insulin deficiency being treated and may be for as long as the life of the subject.

10 In another aspect of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a bile salt to include in the composition based on the ability of the bile salt to increase the solubility of a fatty acid component when the composition has a pH of 8.5 or less.

According to other embodiments of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to lower the precipitation point of a bile salt component in the composition to a pH of 5.5 or less.

According to still other embodiments of the present invention, a method of providing a pharmaceutical composition comprises selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to alter the precipitation characteristics of a bile salt component in the composition.

The present invention will now be described with reference to the following examples. It should be appreciated that these examples are for the purposes of illustrating aspects of the present invention, and do not limit the scope of the invention as defined by the claims.

Examples

30 Example 1

Synthesis of 6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (8)

Hexaethylene glycol monobenzyl ether (1). An aqueous sodium hydroxide solution prepared by dissolving 3.99 g (100 mmol) NaOH in 4 ml water was added slowly to monodispersed hexaethylene glycol (28.175 g, 25 ml, 100 mmol). Benzyl chloride (3.9 g, 30.8 mmol, 3.54 ml) was added and the reaction mixture was heated with stirring to 100°C for 18 hours. The reaction mixture was then cooled, diluted with brine (250 ml) and extracted with methylene chloride (200 ml x 2). The combined organic layers were washed with brine once, dried over Na₂SO₄, filtered and concentrated in vacuo to a dark brown oil. The crude product mixture was purified *via* flash chromatography (silica gel, gradient elution: ethyl acetate to 9/1 ethyl acetate/methanol) to yield 8.099 g (70 %) of monodispersed compound 1 as a yellow oil.

Ethyl 6-methylsulfonyloxyhexanoate (2). A solution of monodispersed ethyl 6-hydroxyhexanoate (50.76 ml, 50.41 g, 227 mmol) in dry dichloromethane (75 ml) was chilled in an ice bath and placed under a nitrogen atmosphere. Triethylamine (34.43 ml, 24.99 g, 247 mmol) was added. A solution of methanesulfonyl chloride (19.15 ml, 28.3 g, 247 mmol) in dry dichloromethane (75 ml) was added dropwise from an addition funnel. The mixture was stirred for three and one half hours, slowly being allowed to come to room temperature as the ice bath melted. The mixture was filtered through silica gel, and the filtrate was washed successively with water, saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo to a pale yellow oil. Final purification of the crude product was achieved by flash chromatography (silica gel, 1/1 hexanes/ethyl acetate) to give the monodispersed compound 2 (46.13 g, 85 %) as a clear, colorless oil. FAB MS: *m/e* 239 (M+H), 193 (M-C₂H₅O).

6-{2-[2-(2-{2-[2-(2-Benzoyloxyethoxy)ethoxy]-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (3). Sodium hydride (3.225 g or a 60 % oil dispersion, 80.6 mmol) was suspended in 80 ml of anhydrous toluene, placed under a nitrogen atmosphere and cooled in an ice bath. A solution of the monodispersed alcohol 9 (27.3 g, 73.3 mmol) in 80 ml dry toluene was added to the NaH suspension. The mixture was stirred at 0°C for thirty minutes, allowed to come to room temperature and stirred for another five hours, during which time the mixture became a clear brown solution. The monodispersed mesylate 10 (19.21 g, 80.6 mmol) in 80 ml dry toluene was added to the NaH/alcohol mixture, and the combined solutions were stirred at room temperature for three days. The reaction mixture was quenched with 50 ml methanol and filtered through basic alumina. The filtrate was

concentrated in vacuo and purified by flash chromatography (silica gel, gradient elution: 3/1 ethyl acetate/hexanes to ethyl acetate) to yield the monodispersed compound 3 as a pale yellow oil (16.52 g, 44 %). FAB MS: *m/e* 515 (M+H).

5 **6-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (4).** Substantially monodispersed benzyl ether 3 (1.03 g, 2.0 mmol) was dissolved in 25 ml ethanol. To this solution was added 270 mg 10 % Pd/C, and the mixture was placed under a hydrogen atmosphere and stirred for four hours, at which time TLC showed the complete disappearance of the starting material. The reaction mixture was
10 filtered through Celite 545 to remove the catalyst, and the filtrate was concentrated in vacuo to yield the monodispersed compound 4 as a clear oil (0.67 g, 79 %). FAB MS: *m/e* 425 (M+H), 447 (M+Na).

6-{2-[2-(2-{2-[2-(2-methylsulfonylethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (5). The monodispersed alcohol 4 (0.835 g, 1.97 mmol) was dissolved in 3.5 ml dry dichloromethane and placed under a nitrogen atmosphere. Triethylamine (0.301 ml, 0.219 g, 2.16 mmol) was added and the mixture was chilled in an ice bath. After two minutes, the methanesulfonyl chloride (0.16 ml, 0.248 g, 2.16 mmol) was added. The mixture was stirred for 15 minutes at 0 °C, then at room temperature for two
20 hours. The reaction mixture was filtered through silica gel to remove the triethylammonium chloride, and the filtrate was washed successively with water, saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 9/1 ethyl acetate/methanol) to give monodispersed compound 5 as a clear oil (0.819 g, 83 %). FAB MS: *m/e* 503 (M+H).

25

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid ethyl ester (6). NaH (88 mg of a 60 % dispersion in oil, 2.2 mmol) was suspended in anhydrous toluene (3 ml) under N₂ and chilled to 0 °C. Monodispersed diethylene glycol monomethyl ether (0.26 ml, 0.26 g, 2.2 mmol) that had been dried *via*
30 azeotropic distillation with toluene was added. The reaction mixture was allowed to warm to room temperature and stirred for four hours, during which time the cloudy grey suspension became clear and yellow and then turned brown. Mesylate 5 (0.50 g, 1.0 mmol) in 2.5 ml dry toluene was added. After stirring at room temperature over night, the reaction was quenched

by the addition of 2 ml of methanol and the resultant solution was filtered through silica gel. The filtrate was concentrated in vacuo and the FAB MS: *m/e* 499 (M+H), 521 (M+Na). Additional purification by preparatory chromatography (silica gel, 19/3 chloroform/methanol) provided the monodispersed compound 6 as a clear yellow oil (0.302 g 5 57 %). FAB MS: *m/e* 527 (M+H), 549 (M+Na).

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid (7). Monodispersed ester 6 (0.25 g, 0.46 mmol) was stirred for 18 hours in 0.71 ml of 1 N NaOH. After 18 hours, the mixture was concentrated in vacuo to 10 remove the alcohol and the residue dissolved in a further 10 ml of water. The aqueous solution was acidified to pH 2 with 2 N HCl and the product was extracted into dichloromethane (30 ml x 2). The combined organics were then washed with brine (25 ml x 2), dried over Na₂SO₄, filtered and concentrated in vacuo to yield the monodispersed compound 15 as a yellow oil (0.147 g, 62 %). FAB MS: *m/e* 499 (M+H), 521 (M+Na).

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (8). Monodispersed acid 7 (0.209 g, 0.42 mmol) was dissolved in 4 ml of dry dichloromethane and added to a dry flask already containing NHS (N-hydroxysuccinimide) (57.8 mg, 0.502 mmol) and EDC (1-(3- 20 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (98.0 mg, 0.502 mmol) under a N₂ atmosphere. The solution was stirred at room temperature overnight and filtered through silica gel to remove excess reagents and the urea formed from the EDC. The filtrate was concentrated in vacuo to provide the activated monodispersed oligomer 8 as a dark yellow oil (0.235 g, 94 %). FAB MS: *m/e* 596 (M+H), 618 (M+Na).

Example 2

Synthesis of Activated MPEG₇-C₈ (14)

Mesylate of triethylene glycol monomethyl ether (9). To a solution of CH₂Cl₂ (100 mL) cooled to 0°C in an ice bath was added monodispersed triethylene glycol monomethyl 30 ether (25 g, 0.15 mol). Then triethylamine (29.5 mL, 0.22 mol) was added and the solution was stirred for 15 min at 0°C, which was followed by dropwise addition of methanesulfonyl chloride (13.8 mL, 0.18 mol, dissolved in 20 mL CH₂Cl₂). The reaction mixture was stirred for 30 min at 0°C, allowed to warm to room temperature, and then stirred for 2 h. The crude

reaction mixture was filtered through Celite (washed CH_2Cl_2 ~200 mL), then washed with H_2O (300 mL), 5% NaHCO_3 (300 mL), H_2O (300 mL), sat. NaCl (300 mL), dried MgSO_4 , and evaporated to dryness. The oil was then placed on a vacuum line for ~2h to ensure dryness and afforded the monodispersed compound **9** as a yellow oil (29.15 g, 80% yield).

5

Heptaethylene glycol monomethyl ether (10). To a solution of monodispersed tetraethylene glycol (51.5 g, 0.27 mol) in THF (1L) was added potassium t-butoxide (14.8 g, 0.13 mol, small portions over ~30 min). The reaction mixture was then stirred for 1h and then **9** (29.15 g, 0.12 mol) dissolved in THF (90 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , ~200 mL) and evaporated to dryness. The oil was then dissolved in HCl (250 mL, 1 N) and washed with ethyl acetate (250 mL) to remove excess **9**. Additional washings of ethyl acetate (125 mL) may be required to remove remaining **9**. The aqueous phase was washed repetitively with CH_2Cl_2 (125 mL volumes) until most of the compound **18** has been removed from the aqueous phase. The first extraction will contain **9**, **10**, and dicoupled side product and should be back extracted with HCl (125 mL, 1N). The organic layers were combined and evaporated to dryness. The resultant oil was then dissolved in CH_2Cl_2 (100 mL) and washed repetitively with H_2O (50 mL volumes) until **10** was removed. The aqueous fractions were combined, total volume 500 mL, and NaCl was added until the solution became cloudy and then was washed with CH_2Cl_2 (2 x 500 mL). The organic layers were combined, dried MgSO_4 , and evaporated to dryness to afford the monodispersed compound **10** as an oil (16.9 g, 41% yield). It may be desirable to repeat one or more steps of the purification procedure to ensure high purity.

8-Bromooctanoate (11). To a solution of monodispersed 8-bromooctanoic acid (5.0 g, 22 mmol) in ethanol (100 mL) was added H_2SO_4 (0.36 mL, 7.5 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. NaHCO_3 (2 x 100 mL), H_2O (100 mL), dried MgSO_4 , and evaporated to dryness to afford a clear oil **11** (5.5 g, 98% yield).

30

MPEG₇-C₈ ester (12). To a solution of the monodispersed compound **10** (3.0 g, 8.8 mmol) in ether (90 mL) was added potassium t-butoxide (1.2 g, 9.6 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of the monodispersed compound **11** (2.4 g, 9.6 mmol), dissolved in ether (10 mL), was added and the reaction mixture was stirred

overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried MgSO_4 , and evaporated to dryness. Column chromatography (Silica, ethyl acetate to ethyl acetate/methanol, 10:1) was performed and afforded the monodispersed compound **12** as a clear oil (0.843 g, 19% yield).

MPEG₇-C₈ acid (13). To the oil of the monodispersed compound **12** (0.70 g, 1.4 mmol) was added 1N NaOH (2.0 mL) and the reaction mixture was stirred for 4h. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl, dried MgSO_4 , and evaporated to dryness to afford the monodispersed compound **13** as a clear oil (0.35 g, 53% yield).

Activation of MPEG₇-C₈ acid. Monodispersed mPEG₇-C₈-acid **13** (0.31g, 0.64 mmol) was dissolved in 3 ml of anhydrous methylene chloride and then solution of N-hydroxysuccinimide (0.079g, 0.69 mmol) and EDCI-HCl (135.6 mg, 0.71 mmol) in anhydrous methylene chloride added. Reaction was stirred for several hours, then washed with 1N HCl, water, dried over MgSO_4 , filtered and concentrated. Crude material was purified by column chromatography, concentrated to afford monodispersed activated MPEG₇-C₈ **14** as a clear oil and dried *via* vacuum.

Example 3

Synthesis of Activated MPEG₇-C₁₀ (19)

10-hydroxydecanoate (15). To a solution of monodispersed 10-hydroxydecanoic acid (5.0 g, 26.5 mmol) in ethanol (100 mL) was added H_2SO_4 (0.43 mL, 8.8 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. NaHCO_3 (2 x 100 mL), H_2O (100 mL), dried MgSO_4 , and evaporated to dryness to afford the monodispersed compound **15** as a clear oil (6.9 g, 98% yield).

30

Mesylate of 10-hydroxydecanoate (16). To a solution of CH_2Cl_2 (27 mL) was added monodispersed 10-hydroxydecanoate **15** (5.6 g, 26 mmol) and cooled to 0°C in an ice bath. Then triethylamine (5 mL, 37 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (2.7 mL, 24 mmol) dissolved in CH_2Cl_2 (3 mL)

was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH₂Cl₂, 80 mL) and the filtrate was washed H₂O (100 mL), 5% NaHCO₃ (2 x 100 mL), H₂O (100 mL), sat. NaCl (100 mL), dried
5 MgSO₄, and evaporated to dryness to afford the monodispersed compound 16 as a yellowish oil (7.42 g, 97% yield).

MPEG₇-C₁₀ Ester (17). To a solution of substantially monodispersed heptaethylene glycol monomethyl ether 10 (2.5 g, 7.3 mmol) in tetrahydrofuran (100 mL) was added
10 sodium hydride (0.194 g, 8.1 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of mesylate of monodispersed 10-hydroxydecanoate 16 (2.4 g, 8.1 mmol), dissolved in tetrahydrofuran (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH₂Cl₂, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed
15 H₂O (2 x 200 mL), dried MgSO₄, evaporated to dryness, chromatographed (silica, ethyl acetate/methanol, 10:1), and chromatographed (silica, ethyl acetate) to afford the monodispersed compound 17 as a clear oil (0.570 g, 15% yield).

MPEG₇-C₁₀ Acid (18). To the oil of monodispersed mPEG₇-C₁₀ ester 17 (0.570 g, 1.1 mmol) was added 1N NaOH (1.6 mL) and the reaction mixture was stirred overnight.
20 The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH₂Cl₂ (2 x 50 mL). The organic layers were combined, washed sat. NaCl (2 x 50 mL), dried MgSO₄, and evaporated to dryness to afford the monodispersed compound 18 as a clear oil (0.340 g, 62% yield).

25

Activation of MPEG₇-C₁₀ Acid. The monodispersed acid 18 was activated using procedures similar to those described above in Example 10 to provide activated MPEG₇-C₁₀ Oligomer 19.

30

Example 4

Synthesis of Activated C₁₈(PEG₆) Oligomer (22)

Synthesis of C₁₈(PEG₆) Oligomer (20). Monodispersed stearoyl chloride (0.7g, 2.31 mmol) was added slowly to a mixture of monodispersed PEG₆ (5 g, 17.7 mmol) and pyridine (0.97g, 12.4 mmol) in benzene. The reaction mixture was stirred for several hours (~5). The

reaction was followed by TLC using ethylacetate/methanol as a developing solvent. Then the reaction mixture was washed with water, dried over MgSO_4 , concentrated and dried *via* vacuum. Purified monodispersed compound **20** was analyzed by FABMS: m/e 549/ M^+H .

5 **Activation of $\text{C}_{18}(\text{PEG}_6)$ Oligomer.** Activation of monodispersed $\text{C}_{18}(\text{PEG}_6)$ oligomer was accomplished in two steps:

1) Monodispersed stearoyl- PEG_6 **20** (0.8 g, 1.46 mmol) was dissolved in toluene and added to a phosgene solution (10 ml, 20 % in toluene) which was cooled with an ice bath. The reaction mixture was stirred for 1 h at 0°C and then for 3 h at room temperature. Then
10 phosgene and toluene were distilled off and the remaining substantially monodispersed stearoyl PEG_6 chloroformate **21** was dried over P_2O_5 overnight.

2) To a solution of monodispersed stearoyl- PEG_6 chloroformate **21** (0.78g, 1.27 mmol) and TEA (128 mg, 1.27 mmol) in anhydrous methylene chloride, N-hydroxy succinimide (NHS) solution in methylene chloride was added. The reaction mixture was
15 stirred for 16 hours, then washed with water, dried over MgSO_4 , filtered, concentrated and dried *via* vacuum to provide the monodispersed activated $\text{C}_{18}(\text{PEG}_6)$ oligomer **22**.

Example 5

Synthesis of Activated $\text{C}_{18}(\text{PEG}_8)$ Oligomer (28)

20 **Tetraethylene glycol monobenzylether (23).** To the oil of monodispersed tetraethylene glycol (19.4 g, 0.10 mol) was added a solution of NaOH (4.0 g in 4.0 mL) and the reaction was stirred for 15 mm. Then benzyl chloride (3.54 mL, 30.8 mmol) was added and the reaction mixture was heated to 100°C and stirred overnight. The reaction mixture was cooled to room temperature, diluted with sat. NaCl (250 mL), and washed CH_2Cl_2 (2 x
25 200 mL). The organic layers were combined, washed sat. NaCl, dried MgSO_4 , and chromatographed (silica, ethyl acetate) to afford the monodispersed compound **23** as a yellow oil (6.21 g, 71% yield).

30 **Mesylate of tetraethylene glycol monobenzylether (24).** To a solution of CH_2Cl_2 (20 mL) was added monodispersed tetraethylene glycol monobenzylether **23** (6.21 g, 22 mmol) and cooled to 0°C in an ice bath. Then triethylamine (3.2 mL, 24 mmol) was added and the reaction mixture was stirred for 15 min at 0°C . Then methanesulfonyl chloride (1.7 mL, 24 mmol) dissolved in CH_2Cl_2 (2 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at

room temperature. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , 80 mL) and the filtrate was washed H_2O (100 mL), 5% NaHCO_3 (2 x 100 mL), H_2O (100 mL), sat. NaCl (100 mL), and dried MgSO_4 . The resulting yellow oil was chromatographed on a pad of silica containing activated carbon (10 g) to afford the monodispersed compound
5 24 as a clear oil (7.10 g, 89% yield).

Octaethylene glycol monobenzylether (25). To a solution of tetrahydrofuran (140 mL) containing sodium hydride (0.43 g, 18 mmol) was added dropwise a solution of monodispersed tetraethylene glycol (3.5 g, 18 mmol) in tetrahydrofuran (10 mL) and the
10 reaction mixture was stirred for 1 h. Then mesylate of monodispersed tetraethylene glycol monobenzylether 24 (6.0 g, 16.5 mmol) dissolved in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed, CH_2Cl_2 , 250 mL) and the filtrate was washed H_2O , dried MgSO_4 , and evaporated to dryness. The resultant oil was chromatographed (silica, ethyl
15 acetate/methanol, 10:1) and chromatographed (silica, chloroform/methanol, 25:1) to afford the monodispersed compound 25 as a clear oil (2.62 g, 34% yield).

Synthesis of Stearate PEG_8 -Benzyl (26). To a stirred cooled solution of monodispersed octaethylene glycol monobenzylether 25 (0.998 g, 2.07 mmol) and pyridine
20 (163.9 mg, 2.07 mmol) was added monodispersed stearoyl chloride (627.7 mg, 2.07 mmol) in benzene. The reaction mixture was stirred overnight (18 hours). The next day the reaction mixture was washed with water, dried over MgSO_4 , concentrated and dried *via* vacuum. Then the crude product was chromatographed on flash silica gel column, using 10% methanol/90% chloroform. The fractions containing the product were combined,
25 concentrated and dried *via* vacuum to afford the monodispersed compound 26.

Hydrogenolysis of Stearate- PEG_8 -Benzyl. To a methanol solution of monodispersed stearate- PEG_8 -Bzl 26 (0.854g 1.138 mmol) Pd/C (10%) (palladium, 10% wt. on activated carbon) was added. The reaction mixture was stirred overnight (18 hours) under
30 hydrogen. Then the solution was filtered, concentrated and purified by flash column chromatography using 10% methanol/90% chloroform, fractions with $R_f=0.6$ collected, concentrated and dried to provide the monodispersed acid 27.

Activation of $\text{C}_{18}(\text{PEG}_8)$ Oligomer. Two step activation of monodispersed stearate-

PEG8 oligomer 27 was performed as described for stearate-PEG₆ in Example 4 above to provide the monodispersed activated C₁₈(PEG₈) oligomer 28.

Example 6

5 **Synthesis of Activated Triethylene Glycol Monomethyl Oligomers**

A solution of toluene containing 20% phosgene (100 ml, approximately 18.7 g, 189 mmol phosgene) was chilled to 0°C under a N₂ atmosphere. Monodispersed mTEG (triethylene glycol, monomethyl ether, 7.8 g, 47.5 mmol) was dissolved in 25 mL anhydrous ethyl acetate and added to the chilled phosgene solution. The mixture was stirred for one
10 hour at 0°C, then allowed to warm to room temperature and stirred for another two and one half hours. The remaining phosgene, ethyl acetate and toluene were removed *via* vacuum distillation to leave the monodispersed mTEG chloroformate as a clear oily residue.

The monodispersed nTEG chloroformate was dissolved in 50 mL of dry dichloromethane to which was added TEA (triethyleamine, 6.62 mL, 47.5 mmol) and NHS
15 (N-hydroxysuccinimide, 5.8 g, 50.4 mmol). The mixture was stirred at room temperature under a dry atmosphere for twenty hours during which time a large amount of white precipitate appeared. The mixture was filtered to remove this precipitate and concentrated *in vacuo*. The resultant oil was taken up in dichloromethane and washed twice with cold deionized water, twice with 1N HCl and once with brine. The organics were dried over
20 MgSO₄, filtered and concentrated to provide the monodispersed title compound as a clear, light yellow oil. If necessary, the NHS ester could be further purified by flash chromatography on silica gel using EtOAc as the elutant.

Example 7

25 **Synthesis of Activated Palmitate-TEG Oligomers**

Monodispersed palmitic anhydride (5 g; 10 mmol) was dissolved in dry THF (20 mL) and stirred at room temperature. To the stirring solution, 3 mol excess of pyridine was added followed by monodispersed triethylene glycol (1.4 mL). The reaction mixture was stirred for 1 hour (progress of the reaction was monitored by TLC; ethyl acetate-chloroform; 3:7). At
30 the end of the reaction, THF was removed and the product was mixed with 10% H₂SO₄ acid and extracted ethyl acetate (3 x 30 mL). The combined extract was washed sequentially with water, brine, dried over MgSO₄, and evaporated to give monodispersed palmitate-TEG oligomers.

A solution of N,N'-disuccinimidyl carbonate (3 mmol) in DMF (~10 mL) is added to

a solution of the monodispersed palmitate-TEG oligomers (1 mmol) in 10 mL of anhydrous DMF while stirring. Sodium hydride (3 mmol) is added slowly to the reaction mixture. The reaction mixture is stirred for several hours (e.g., 5 hours). Diethyl ether is added to precipitate the monodispersed activated title oligomer. This process is repeated 3 times and the product is finally dried.

Example 8

Synthesis of Activated Hexaethylene Glycol Monomethyl Oligomers

Monodispersed activated hexaethylene glycol monomethyl ether was prepared analogously to that of monodispersed triethylene glycol in Example 6 above. A 20% phosgene in toluene solution (35 mL, 6.66 g, 67.4 mmol phosgene) was chilled under a N₂ atmosphere in an ice/salt water bath. Monodispersed hexaethylene glycol (1.85 mL, 2.0 g, 6.74 mmol) was dissolved in 5 mL anhydrous EtOAc and added to the phosgene solution *via* syringe. The reaction mixture was kept stirring in the ice bath for one hour, removed and stirred a further 2.5 hours at room temperature. The phosgene, EtOAc, and toluene were removed by vacuum distillation, leaving monodispersed methyl hexaethylene glycol chloroformate as a clear, oily residue.

The monodispersed chloroformate was dissolved in 20 mL dry dichloromethane and placed under a dry, inert atmosphere. Triethylamine (0.94 mL, 0.68 g, 6.7 mmol) and then NHS (N-hydroxy succinimide, 0.82 g, 7.1 mmol) were added, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was filtered through silica gel to remove the white precipitate and concentrated *in vacuo*. The residue was taken up in dichloromethane and washed twice with cold water, twice with 1 N HCl and once with brine. The organics were dried over Na₂SO₄, filtered and concentrated. Final purification was done via flash chromatography (silica gel, EtOAc) to obtain the activated monodispersed hexaethylene monomethyl ether.

Example 9

Synthesis of Activated Heptaethylene Glycol Monomethyl Ether

8-Methoxy-1-(methylsulfonyl)oxy-3,6-dioxaoctane (29). A solution of monodispersed triethylene glycol monomethyl ether molecules (4.00 mL, 4.19 g, 25.5 mmol) and triethylamine (4.26 mL, 3.09 g, 30.6 mmol) in dry dichloromethane (50 mL) was chilled in an ice bath and placed under a nitrogen atmosphere. A solution of methanesulfonyl chloride

(2.37 mL, 3.51 g, 30.6 mmol) in dry dichloromethane (20 mL) was added dropwise from an addition funnel. Ten minutes after the completion of the chloride addition, the reaction mixture was removed from the ice bath and allowed to come to room temperature. The mixture was stirred for an additional hour, at which time TLC (CHCl₃ with 15% MeOH as the elutant) showed no remaining triethylene glycol monomethyl ether.

The reaction mixture was diluted with another 75 mL of dichloromethane and washed successively with saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo to give a monodispersed mixture of compounds 29 as a clear oil (5.31 g, 86%).

10

Heptaethylene glycol mono methyl ether (30). To a stirred solution of monodispersed tetraethylene glycol (35.7 mmol) in dry DMF (25.7 mL), under N₂ was added in portion a 60% dispersion of NaH in mineral oil, and the mixture was stirred at room temperature for 1 hour. To the resulting sodium salt of the tetraethylene glycol was added a solution of monodispersed mesylate 29 (23.36) in dry DMF (4 ml) in a single portion, and the mixture was stirred at room temperature for 3.5 hours. Progress of the reaction was monitored by TLC (12% CH₃OH-CHCl₃). The reaction mixture was diluted with an equal amount of 1N HCl, and extracted with ethyl acetate (2 x 20 ml) and discarded. Extraction of aqueous solution and work-up gave monodispersed heptaethylene glycol monomethyl ether 30 (82 -84% yield). Oil; Rf 0.46 (methanol : chloroform = 3:22); MS m/z calc'd for C₁₅H₃₂O₈ 340.21 (M⁺+1), found 341.2.

20

Activation of heptaethylene glycol monomethyl ether. Monodispersed heptaethylene glycol monomethyl ether 30 is activated by a procedure similar to that used in Example 6 above to activate triethylene glycol monomethyl ether to provide the activated heptaethylene glycol monomethyl ether.

25

Example 10

Synthesis of Activated Decaethylene Glycol Monomethyl Ether (33)

20-methoxy-1-(methylsulfonyl)oxy-3,6,9,12,15,18-hexaoxaicosane (31).

30

Monodispersed compound 31 was obtained in quantitative yield from compound 30 and methanesulfonyl chloride as described for 29 in Example 9 above, as an oil; Rf 0.4 (ethyl acetate : acetonitrile = 1:5); MS m/z calc'd for C₁₇H₃₇O₁₀ 433.21 (M⁺+1), found 433.469.

Decaethylene glycol monomethyl ether (32). Monodispersed compound 32 was prepared from compound 31 and monodispersed triethylene glycol using the procedure described above in Example 17. Oil; Rf 0.41 (methanol: chloroform = 6:10); MS m/z calc'd for C₂₁H₄₄O₁₁ 472.29 (M⁺+1), found 472.29.

5

Activation of decaethylene glycol monomethyl ether. Monodispersed decaethylene glycol monomethyl ether 32 is activated by a procedure similar to that used in Example 6 above to activate triethylene glycol monomethyl ether to provide the activated decaethylene glycol monomethyl ether 33.

10

Example 11

HIM2 Oral Liquid Process

A general procedure to manufacture an oral liquid pharmaceutical composition of the present invention is shown below: The process involves making a premix without the drug, filtering the premix, then adding the premix and drug solution together.

15

Quantitative Composition of HIM2 Oral Liquid, 6 mg/mL

Excipient	Composition		Quantity per Batch (g)
	%w/v	mg/mL	
HIM2	0.6	6	6.0 ¹
Sodium Cholate	3.0	30	30.0
Oleic Acid, NF	1.0	10	10.0
Sucralose, 25%	0.8	8	8.0
Strawberry Flavor	0.4	4	4.0
Capric Acid	0.5	5	5.0
Lauric Acid	0.5	5	5.0
Citric Acid Anhydrous, USP	6.72	67.2	67.2
Trolamine, NF	5.22	52.2	52.2
Tromethamine, USP	4.24	42.4	42.4
Sodium Hydroxide, NF	1.88	18.8	18.8
Sodium Hydroxide, 5 N	QS	QS	QS
Hydrochloric Acid, 5N	QS	QS	QS
Sterile Water for Irrigation, USP	QS	QS	QS
Total	100%	1.0 mL	1077.4 g

20 1. Weight adjusted for protein content.

Preparation of Premix for HIM2 Oral Liquid

1. Add 94.3% of the tromethamine and the trolamine, citric acid and sodium hydroxide (NF) to 350 g sterile water for irrigation and stir until completely dissolved.
- 5 2. Moderately heat and maintain the temperature through steps 3 & 4, below.
3. Add the sodium cholate to step 2 and stir until dissolved.
4. Add the oleic acid, capric acid, lauric acid, sucralose solution and strawberry flavor to step 3 and stir until dissolved.
5. Adjust the temperature to approximately room temperature.
- 10 6. Adjust the pH of step 5, if necessary, to 7.8 ± 0.1 using 5N sodium hydroxide or 5N hydrochloric acid.
7. QS to the pre-mix batch weight with sterile water for irrigation.
8. Filter the step 7 product.

15 Preparation of HIM2 Oral Liquid, 6 mg/mL

1. Dispense the required quantity of the Premix for HIM2 Oral Liquid and continue stirring while performing steps 2 through 4 below.
2. Add the remaining tromethamine to 140 g of the sterile water for irrigation and stir
20 until dissolved.
3. Adjust the pH of step 2, if necessary, to 7.7 ± 0.2 using 5N sodium hydroxide or 5N hydrochloric acid.
4. Filter the step 3 liquid.
5. Add all of the HIM2 to step 4 and stir until completely dissolved.
- 25 6. Add all of step 5 to step 1 and stir.
7. Adjust the temperature to approximately room temperature, if necessary
8. Adjust the pH of step 7, if necessary, to 7.6-7.9 using 5N sodium hydroxide or 5N hydrochloric acid.

30

Example 12**HIM2 Oral Tablet Process**

A general procedure to manufacture an oral tablet formulation of the present invention is shown below: The process involves making a lyophilized powder, adding tableting excipients and compressing.

Quantitative Composition of HIM2 Oral Tablets, 10 mg

Excipient Lyo Portion	Quantity per Batch (g)
HIM2	2.50 ¹
Sodium Cholate	30.0
Oleic Acid, NF	10.0
Sucralose, 25%	8.0
Strawberry Flavor	4.0
Capric Acid	5.0
Lauric Acid	5.0
Citric Acid Anhydrous, USP	67.2
Trolamine, NF	52.2
Tromethamine, USP	42.4
Sodium Hydroxide, NF	18.8
Sodium Hydroxide, 5 N	QS
Hydrochloric Acid, 5N	QS
Sterile Water for Irrigation, USP	QS
Total	1077.4 g

Tablet Portion	
Lyo Portion	127.6
Citric Acid	29.7
Sodium Citrate dihydrate	84.2
Tris Base (tris(hydroxymethyl)aminomethane)	106.7
Microcrystalline Cellulose	24.8
Explotab	9.4
Total	382.3

- 5 1. Weight adjusted for protein content.

Procedure:

1. Dispense the required ingredients with the exception of the Sterile Water for Irrigation, USP,
- 10 2. Dispense 1500 g of Sterile Water for Irrigation, USP, and add to the processing vessel above.
3. Add the following ingredients to step 2 and mix until dissolved completely:
 - a. all of the Sodium Cholate
 - b. all of the Dibasic Sodium Phosphate Heptahydrate, USP

4. Add the following ingredients to step 3 and mix vigorously.
 - a. All of the Capric Acid
 - b. All of the Lauric Acid
 - c. All of the Sodium Hydroxide, NF (Note: Heat will be generated by the
5 addition of Sodium Hydroxide, NF.)
5. Adjust the step 4 solution to a temperature between 45°C and 50°C, and mix until a clear solution results.
6. Cool to room temperature and, if necessary, adjust the step 5 pH to 7.2 – 7.8 using Sodium Hydroxide, 1N, or Hydrochloric Acid, 1N.
- 10 7. Add all of the HIM2 PEG 7 to step 6 and mix vigorously until a clear solution results.
8. Determine the amount of additional Sterile Water for Irrigation, USP, to add (if necessary)
9. Lyophilize this solution until a white amorphous powder results.
10. Blend the lyo portion with the tableting excipients.
- 15 11. Compress on a tablet press to achieve desired size, shape and hardness.

Example 13

Liquid oral pharmaceutical compositions formulated in accordance with Example 11 above were administered to male CF-1 mice (~20-25 g). The animals were fasted overnight and deprived of food during the experiment. Water was provided ad libitum. The mice were
20 maintained in cages with 5 animals per cage and kept in a room with a 12:12 L:D cycle (6:00 a.m.-6:00 p.m.). The mice were tested in groups of 5 animals per dose. Each group of mice (N=5) received either insulin conjugate-075, insulin conjugate-076, insulin-conjugate-084, insulin-conjugate-098, insulin-conjugate-101, insulin conjugate-106 or HIM2 orally at 1.25
25 and 2.5 mg/kg.

The insulin conjugates and HIM2 were provided at concentrations of 0.125 and 0.25 mg/ml. The dosing volume was 10.0 mL/kg. Total doses (each compound) animals received were 1.25 and 2.5 mg/kg. Oral doses were administered using a gavaging needle (Popper gavage needle for mice #20; 5 cm from hub to bevel). Effect of the various conjugates on
30 blood glucose level is illustrated in Figures 7, 8, and 9.

Example 14

Liquid oral pharmaceutical compositions formulated in accordance with Example 11 above were administered to healthy human volunteers in a 4-way crossover study both pre-prandial and post-prandial. The insulin conjugate HIM2 was provided at concentrations of 0.125, 0.25 and 0.5 mg/ml and these were compared to baseline values where no dosing occurred. 20 mL oral doses were provided followed by 70 mL of water.

For the pre-prandial phase (fasted), subjects were fasted overnight and a single dose was administered in the morning. If blood glucose levels fell below 50 mg/dL, the subjects were rescued with a dextrose infusion. The effect of the conjugate HIM2 on blood glucose levels is illustrated in Figure 1. HIM2 plasma levels (expressed in insulin equivalents) are shown in Figure 2. The glucose/dose response and HIM2/dose response is shown in Figure 3.

For the post-prandial phase (fed), subjects received a single dose followed 15 minutes later by a meal. If blood glucose levels fell below 50 mg/dL, the subjects were rescued with a dextrose infusion. The effect of the conjugate HIM2 on blood glucose levels is illustrated in Figure 4. HIM2 plasma levels (expressed in insulin equivalents) are shown in Figure 5. The glucose/dose response and HIM2/dose response is shown in Figure 6.

The present invention has been described herein with reference to its preferred embodiments. These embodiments do not serve to limit the invention, but are set forth for illustrative purposes. The scope of the invention is defined by the claims that follow.

That Which is Claimed is:

1. A pharmaceutical composition comprising:
an insulin drug-oligomer conjugate comprising an insulin drug covalently coupled to
5 an oligomeric moiety;
between 0.1 and 15% (w/v) of a fatty acid component;
between 0.1 and 15% (w/v) of a bile salt component;
wherein the fatty acid component and the bile salt component are present in a weight-to-
weight ratio of between 1:5 and 5:1.
10
2. The pharmaceutical composition according to claim 1, wherein the fatty acid
component and the bile salt component are present in a weight ratio of between 1:2 and 2:1.
3. The pharmaceutical composition according to claim 1, wherein the bile salt
15 component comprises a pharmaceutically acceptable salt of cholic acid.
4. The pharmaceutical composition according to claim 1, wherein the bile salt
component is sodium cholate.
- 20 5. The pharmaceutical composition according to any one of claims 1 to 4,
wherein the fatty acid component comprises a medium-chain fatty acid component and a
long-chain fatty acid component.
6. The pharmaceutical composition according to any one of claims 1 to 4,
25 wherein the pH of the composition is between 6.2 and 9.0.
7. The pharmaceutical composition according to any one of claims 1 to 4,
wherein the insulin drug is an insulin polypeptide.
- 30 8. The pharmaceutical composition of according to claim 7, wherein the insulin
polypeptide is human insulin.
9. The pharmaceutical composition according to claim 8, wherein the oligomeric
moiety is coupled to the lysine at the B29 position of the human insulin.

10. The pharmaceutical composition according to claim 7, wherein the insulin polypeptide is an insulin analog selected from the group consisting of Gly^{A21} insulin, human; Gly^{A21} Gln^{B3} insulin, human; Ala^{A21} insulin, human; Ala^{A21} Gln^{B3} insulin, human; Gln^{B3} insulin, human; Gln^{B30} insulin, human; Gly^{A21} Glu^{B30} insulin, human; Gly^{A21} Gln^{B3} Glu^{B30} insulin, human; Gln^{B3} Glu^{B30} insulin, human; Asp^{B28} insulin, human; Lys^{B28} insulin, human; Leu^{B28} insulin, human; Val^{B28} insulin, human; Ala^{B28} insulin, human; Asp^{B28} Pro^{B29} insulin, human; Lys^{B28} Pro^{B29} insulin, human; Leu^{B28} Pro^{B29} insulin, human; Val^{B28} Pro^{B29} insulin, human; Ala^{B28} Pro^{B29} insulin, human.
11. The pharmaceutical composition according to any one of claims 1 to 4, wherein the insulin drug-oligomer conjugate is present as a substantially monodispersed mixture.
12. The pharmaceutical composition according to any one of claims 1 to 4, wherein the insulin drug-oligomer conjugate is present as a monodispersed mixture.
13. The pharmaceutical composition according to any one of claims 1 to 4, further comprising a buffering component.
14. The pharmaceutical composition according to claim 13, wherein the buffering component comprises tris-base and trolamine.
15. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pharmaceutical composition is a solid dosage pharmaceutical composition.
16. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pharmaceutical composition is a liquid pharmaceutical composition.
17. The pharmaceutical composition according to claim 16, wherein the liquid pharmaceutical composition is suitable for oral administration.
18. The pharmaceutical composition according to claim 16, wherein the liquid pharmaceutical composition is suitable for parenteral administration.

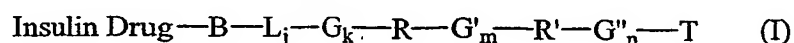
19. The pharmaceutical composition according to any one of claims 1 to 4, wherein the fatty acid component is present in an amount sufficient to lower the precipitation point of the bile salt compared to a precipitation point of the bile salt if the fatty acid component were not present in the pharmaceutical composition, and wherein the bile salt component is present in an amount sufficient to lower the solubility point of the fatty acid compared to a solubility point of the fatty acid if the bile salt were not present in the pharmaceutical composition.

20. The pharmaceutical composition according to any one of claims 1 to 4, wherein the fatty acid component is present in a first amount such that, at the precipitation point of the bile salt, the bile salt precipitates as first bile salt particles that, upon a return to a pH above the precipitation point of the bile salt, re-solubilize more quickly than second bile salt particles that would have precipitated if the fatty acid component were not present in the composition.

21. The pharmaceutical composition according to any one of claims 1 to 4, wherein the bile salt component is present in an amount such that the fatty acid is soluble at a pH of 8.2, and wherein the fatty acid component is present in an amount such that the bile salt remains in solution at a pH of 5.5.

22. The pharmaceutical composition according to any one of claims 1 to 4, wherein the oligomeric moiety comprises a hydrophilic moiety and a lipophilic moiety.

23. The pharmaceutical composition according to any one of claims 1 to 4, wherein the insulin drug-oligomer conjugate comprises the structure of Formula I:



wherein:

B is a bonding moiety;

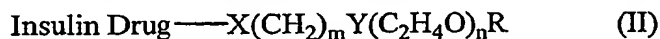
L is a linker moiety;

G, G' and G'' are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and
j, k, m and n are individually 0 or 1.

24. The pharmaceutical composition according to any one of claims 1 to 4,
5 wherein the insulin drug-oligomer conjugate comprises the structure of Formula II:



wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-
carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea
10 moiety or a covalent bond;

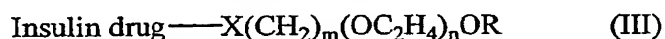
Y is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-
carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea
moiety or a covalent bond;

m is between 1 and 24;

15 n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a
fatty acid moiety.

25. The pharmaceutical composition according to any one of claims 1 to 4,
20 wherein the insulin drug-oligomer conjugate comprises the structure of Formula III:



wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, thio-
carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea
25 moiety, or a covalent bond;

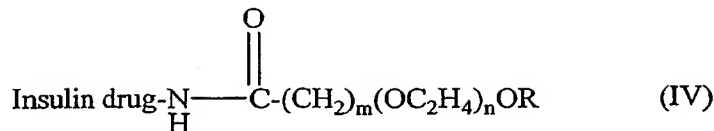
m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a
fatty acid moiety.

30

26. The pharmaceutical composition according to any one of claims 1 to 4,
wherein the insulin drug-oligomer conjugate comprises the structure of Formula IV:



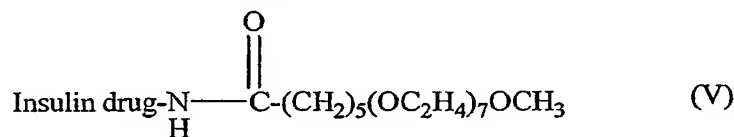
wherein:

m is between 1 and 24;

n is between 1 and 50; and

5 R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

27. The pharmaceutical composition according to any one of claims 1 to 4, wherein the insulin drug-oligomer conjugate comprises the structure of Formula V:



10

28. A method of treating an insulin deficiency in a subject in need of such treatment, said method comprising administering to the subject a pharmaceutical composition comprising (a) a therapeutically effective amount of an insulin drug-oligomer conjugate that
15 comprises an insulin drug covalently coupled to an oligomeric moiety; (b) between 0.1 and 15% (w/v) of a fatty acid component comprising a fatty acid; and (c) between 0.1 and 15% (w/v) of a bile salt component comprising a bile salt, wherein the fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1.

20 29. The method according to claim 28, wherein the fatty acid component and the bile salt component are present in a weight ratio of between 1:2 and 2:1.

30. The method according to claim 28, wherein the bile salt component comprises a pharmaceutically acceptable salt of cholic acid.

25

31. The method according to claim 28, wherein the bile salt component is sodium cholate.

32. The method according to any one of claims 28 to 31, wherein the fatty acid component comprises a medium-chain fatty acid component and a long-chain fatty acid component.

5 33. The method according to any one of claims 28 to 31, wherein the pH of the composition is between 6.2 and 9.0.

34. The method according to any one of claims 28 to 31, wherein the insulin drug is an insulin polypeptide.

10

35. The method according to claim 34, wherein the insulin polypeptide is human insulin.

36. The method according to claim 35, wherein the oligomeric moiety is coupled
15 to the lysine at the B29 position of the human insulin.

37. The method according to claim 34, wherein the insulin polypeptide is an insulin analog selected from the group consisting of Gly^{A21} insulin, human; Gly^{A21} Gln^{B3} insulin, human; Ala^{A21} insulin, human; Ala^{A21} Gln^{B3} insulin, human; Gln^{B3} insulin, human; Gln^{B30} insulin, human; Gly^{A21} Glu^{B30} insulin, human; Gly^{A21} Gln^{B3} Glu^{B30} insulin, human; Gln^{B3} Glu^{B30} insulin, human; Asp^{B28} insulin, human; Lys^{B28} insulin, human; Leu^{B28} insulin, human; Val^{B28} insulin, human; Ala^{B28} insulin, human; Asp^{B28} Pro^{B29} insulin, human; Lys^{B28} Pro^{B29} insulin, human; Leu^{B28} Pro^{B29} insulin, human; Val^{B28} Pro^{B29} insulin, human; Ala^{B28} Pro^{B29} insulin, human.
20

25

38. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate is present as a substantially monodispersed mixture.

39. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate is present as a monodispersed mixture.
30

40. The method according to any one of claims 28 to 31, further comprising a buffering component.

41. The method according to any one of claims 28 to 31, wherein the buffering component comprises tris-base and trolamine.

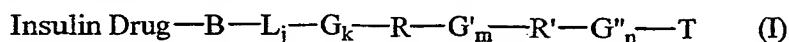
42. The method according to any one of claims 28 to 31, wherein the
5 pharmaceutical composition is a solid dosage pharmaceutical composition.

43. The method according to any one of claims 28 to 31, wherein the pharmaceutical composition is a liquid pharmaceutical composition.

10 44. The method according to any one of claims 28 to 31, wherein the method comprises orally administering the pharmaceutical composition.

45. The method according to any one of claims 28 to 31, wherein the oligomeric moiety comprises a hydrophilic moiety and a lipophilic moiety.
15

46. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate comprises the structure of Formula I:



wherein:

20 B is a bonding moiety;

L is a linker moiety;

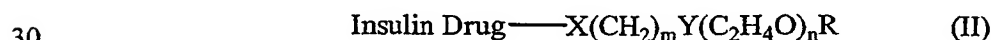
G, G' and G'' are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

25 T is a terminating moiety; and

j, k, m and n are individually 0 or 1.

47. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate comprises the structure of Formula II:



wherein:

X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

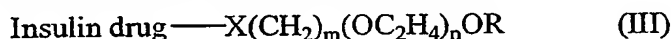
Y is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, a thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

48. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate comprises the structure of Formula III:



wherein:

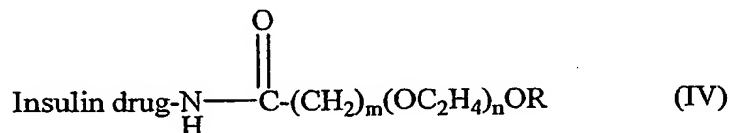
X is an ester moiety, a thio-ester moiety, an ether moiety, a carbamate moiety, thio-carbamate moiety, a carbonate moiety, a thio-carbonate moiety, an amide moiety, a urea moiety, or a covalent bond;

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

49. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate comprises the structure of Formula IV:



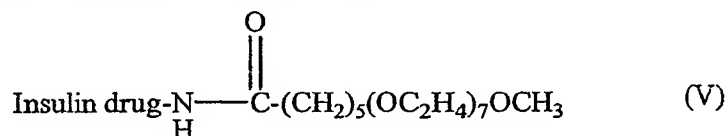
wherein:

m is between 1 and 24;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety.

50. The method according to any one of claims 28 to 31, wherein the insulin drug-oligomer conjugate comprises the structure of Formula V:



5

51. A method of providing a pharmaceutical composition, said method comprising:

selecting an amount of a bile salt to include in the composition based on the ability of the bile salt to increase the solubility of a fatty acid component when the composition has a pH of 8.5 or less.

10

52. A method of providing a pharmaceutical composition, said method comprising:

selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to lower the precipitation point of a bile salt component in the composition to a pH of 5.5 or less.

15

53. A method of providing a pharmaceutical composition, said method comprising:

selecting an amount of a fatty acid to include in the composition based on the ability of the fatty acid to alter the precipitation characteristics of a bile salt component in the composition.

20

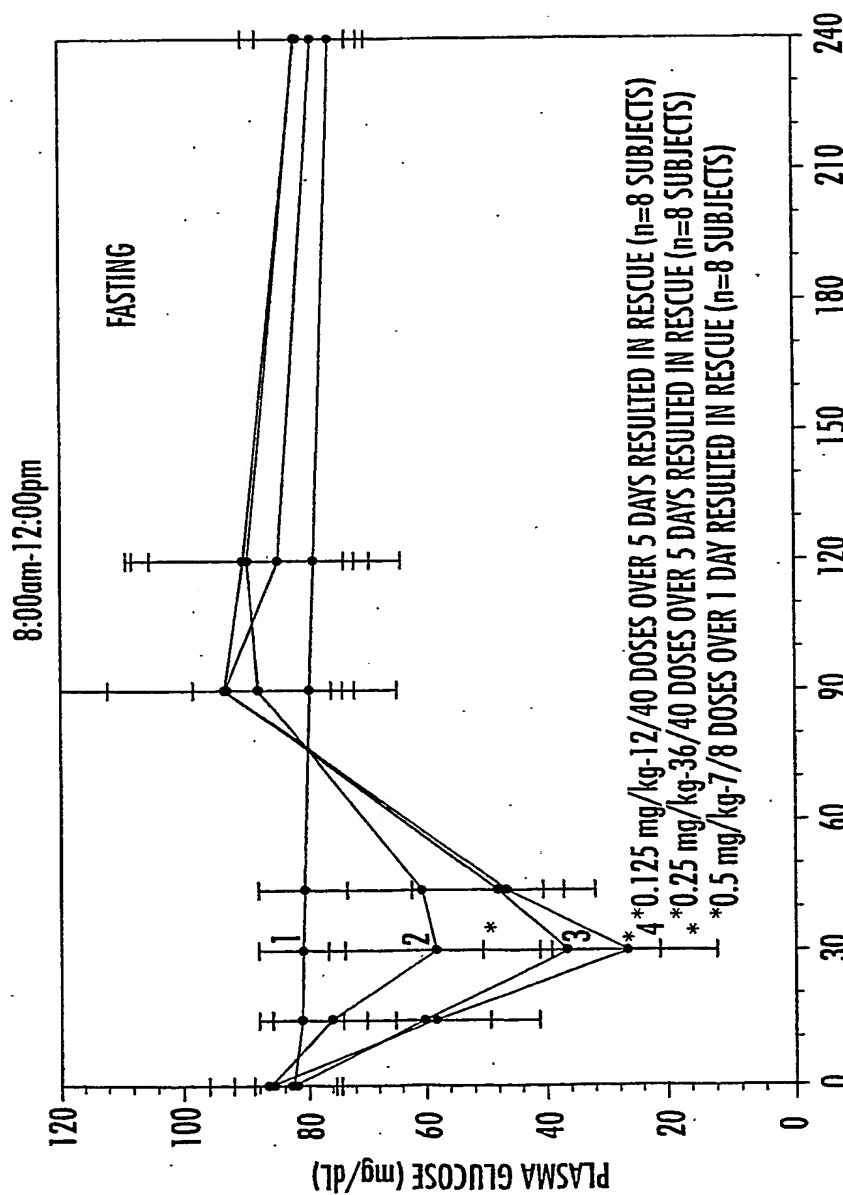


FIG. 1.

2/9

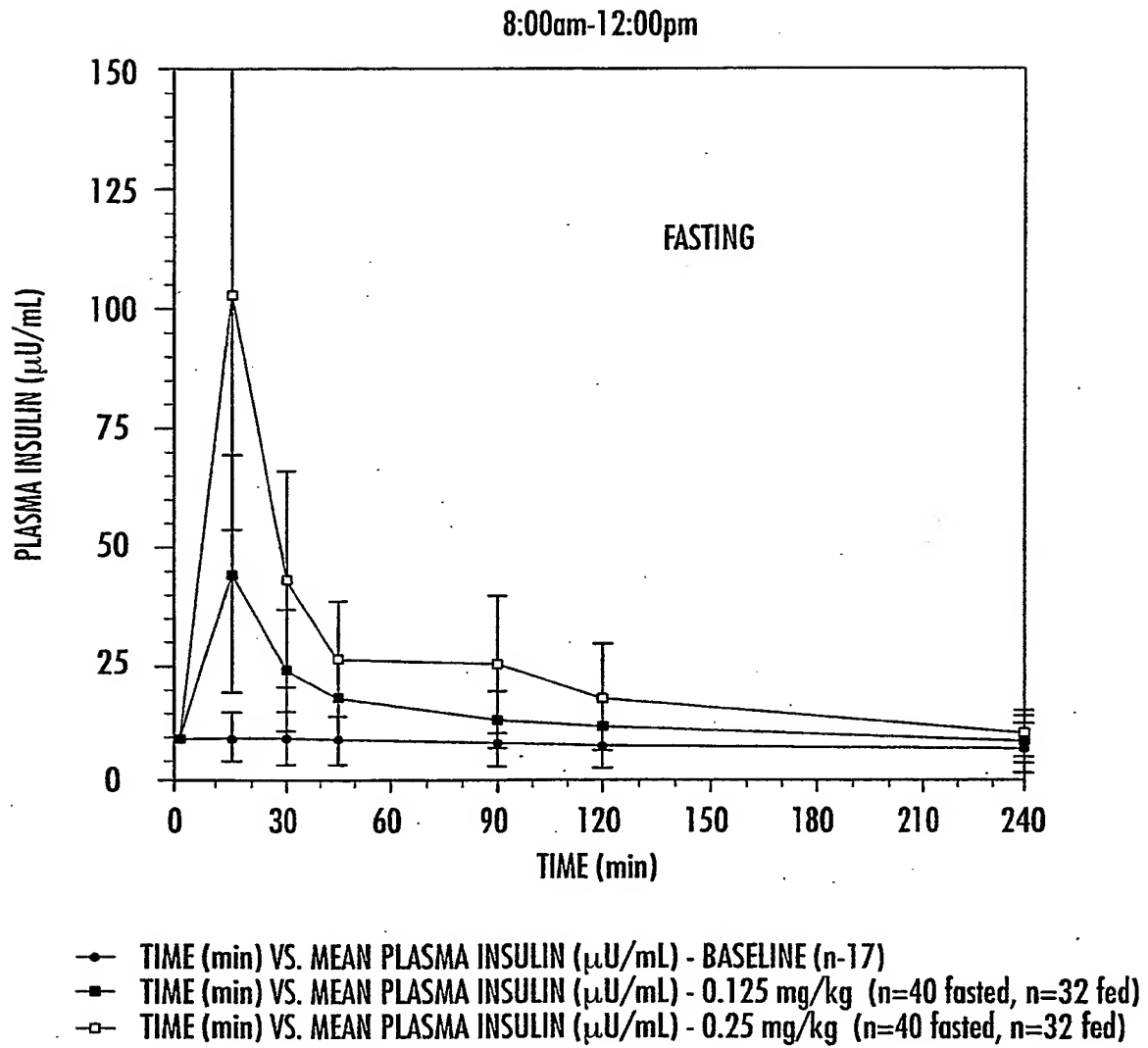


FIG. 2.

3/9

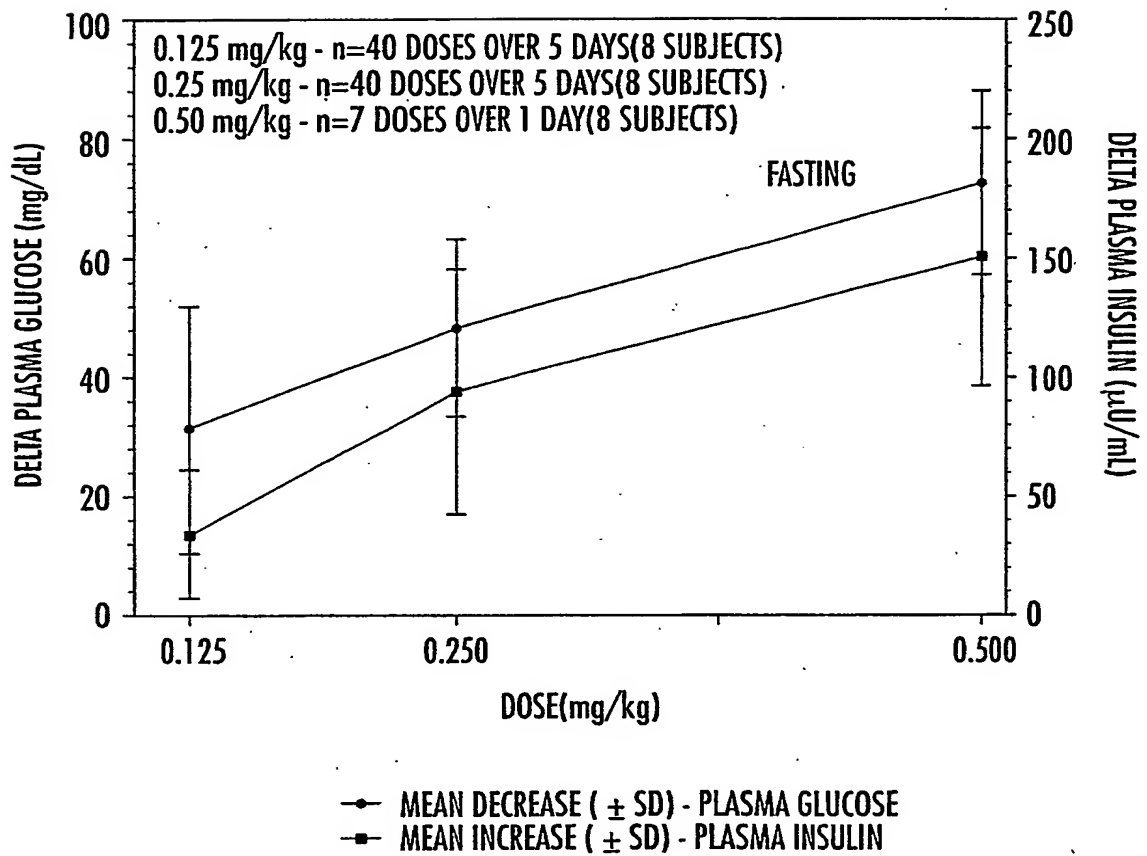


FIG. 3.

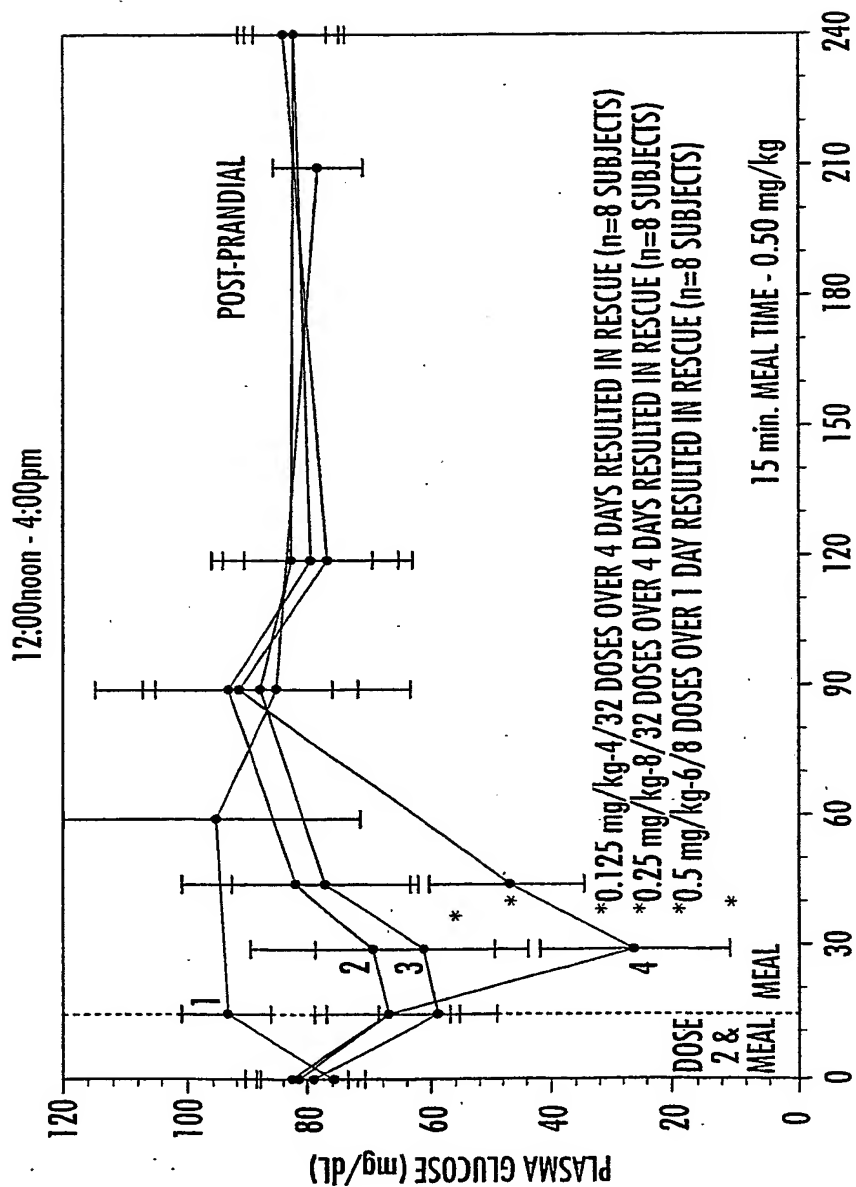
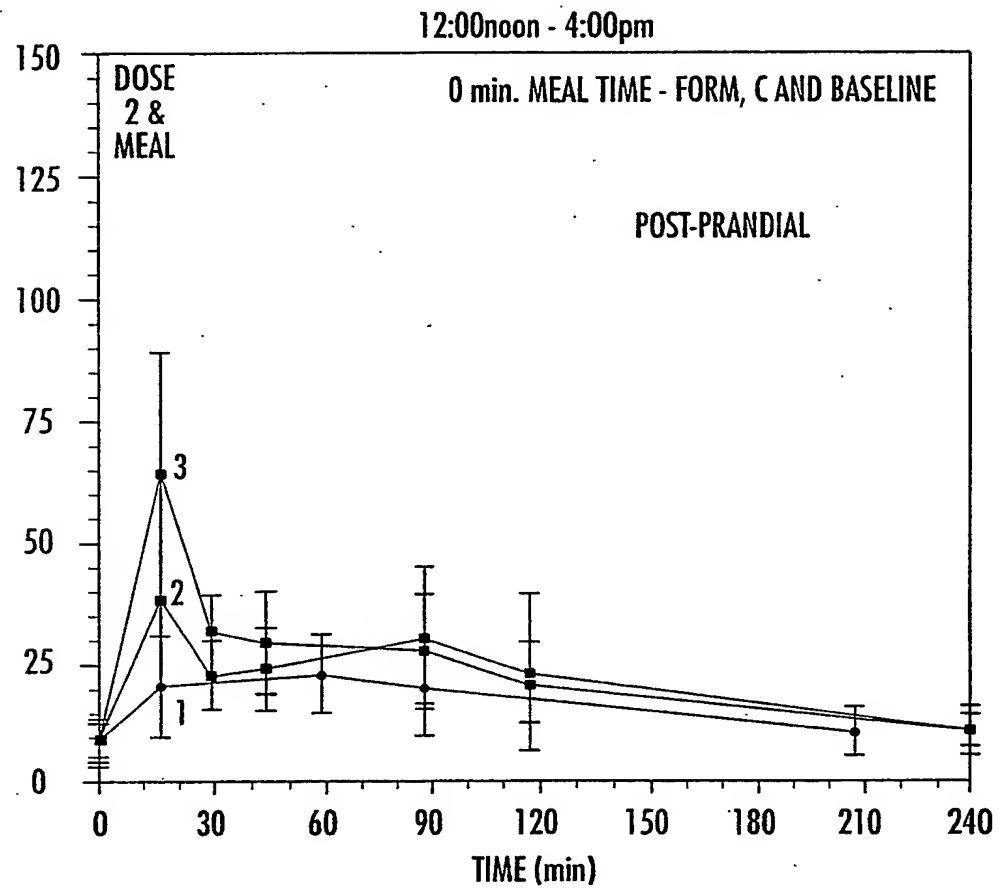


FIG. 4.

5/9



- 1 TIME (min) VS. MEAN PLASMA INSULIN (μ U/mL) - BASELINE (n=17)
 2 TIME (min) VS. MEAN PLASMA INSULIN (μ U/mL) - 0.125 mg/kg (n=40 fasted, n=32 fed)
 3 TIME (min) VS. MEAN PLASMA INSULIN (μ U/mL) - 0.25 mg/kg (n=40 fasted, n=32 fed)

FIG. 5.

6/9

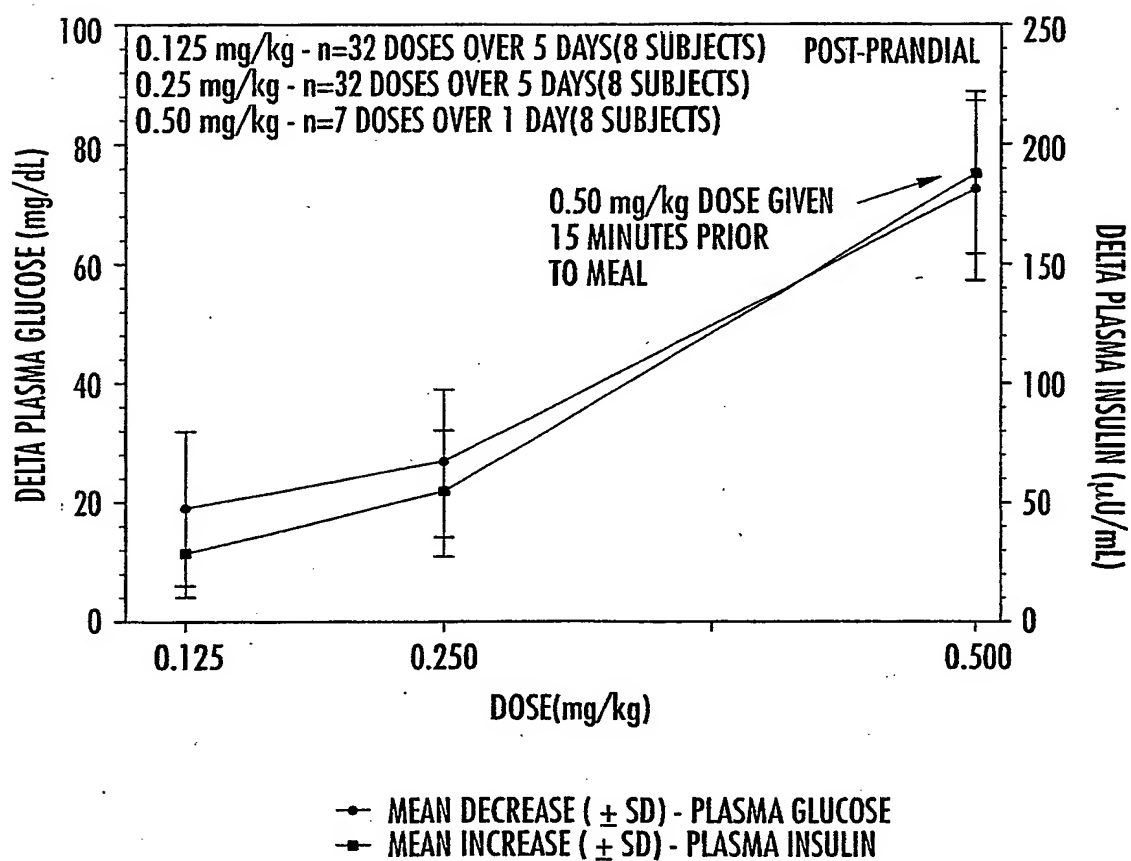


FIG. 6.

7/9

EFFECT OF HIM2, -[076] AND -[084] ON BLOOD GLUCOSE AFTER
ORAL ADMINISTRATION IN MICE (1.25 AND 2.5 mg/kg)
(3% CHOLATE, 1% OLEATE, 0.5% LAURATE, 0.5% CAPRATE
IN LIQUID BUFFER)

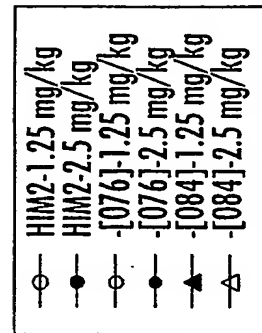
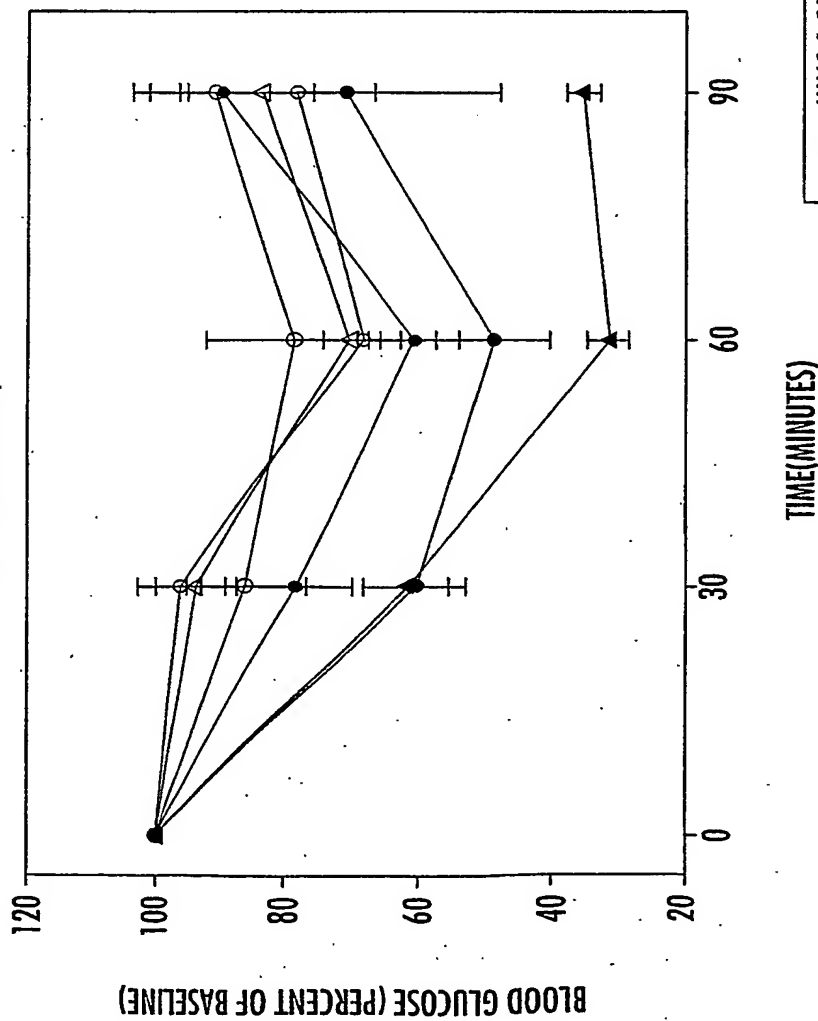


FIG. 7.

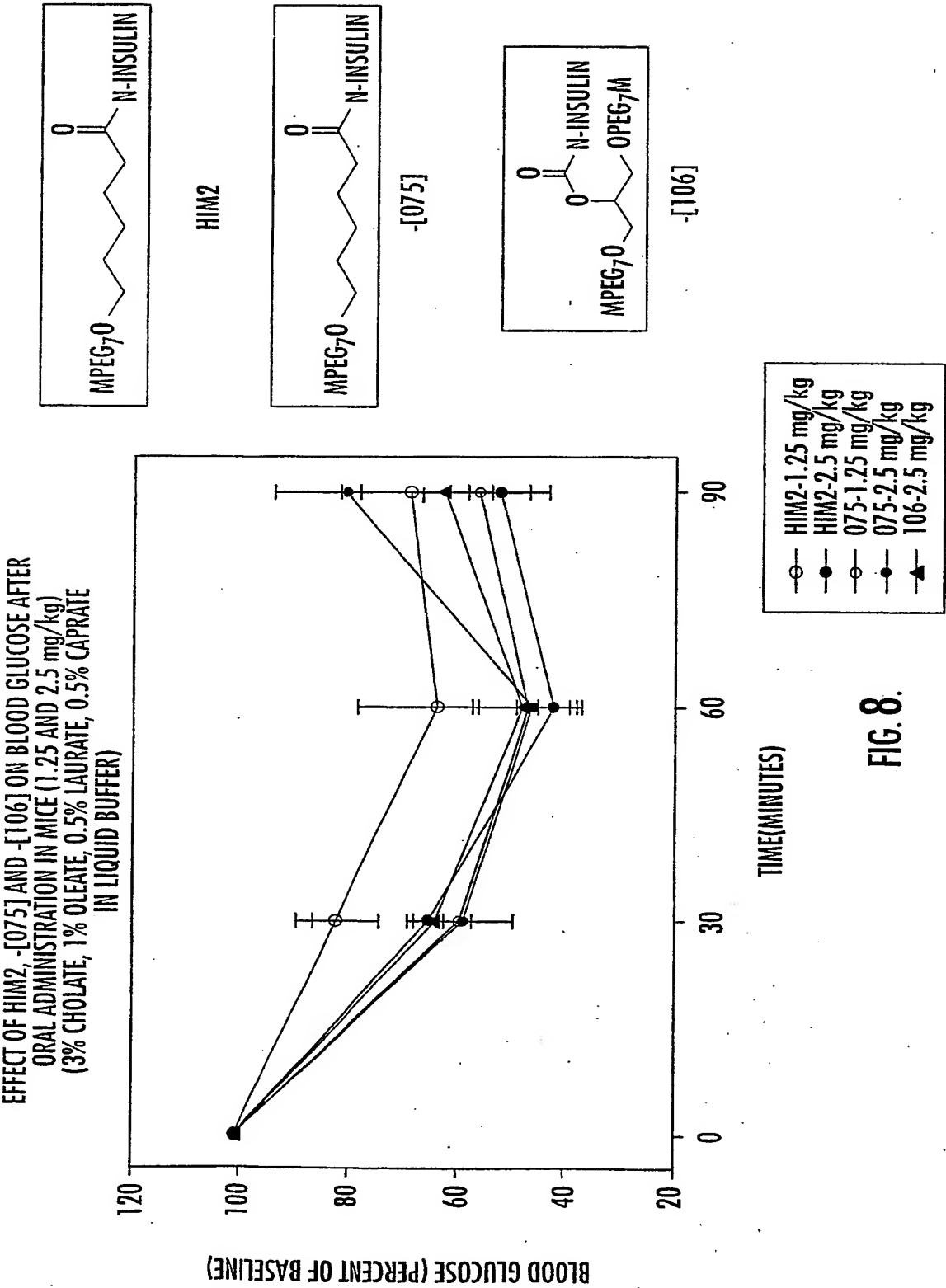


FIG. 8.

9/9

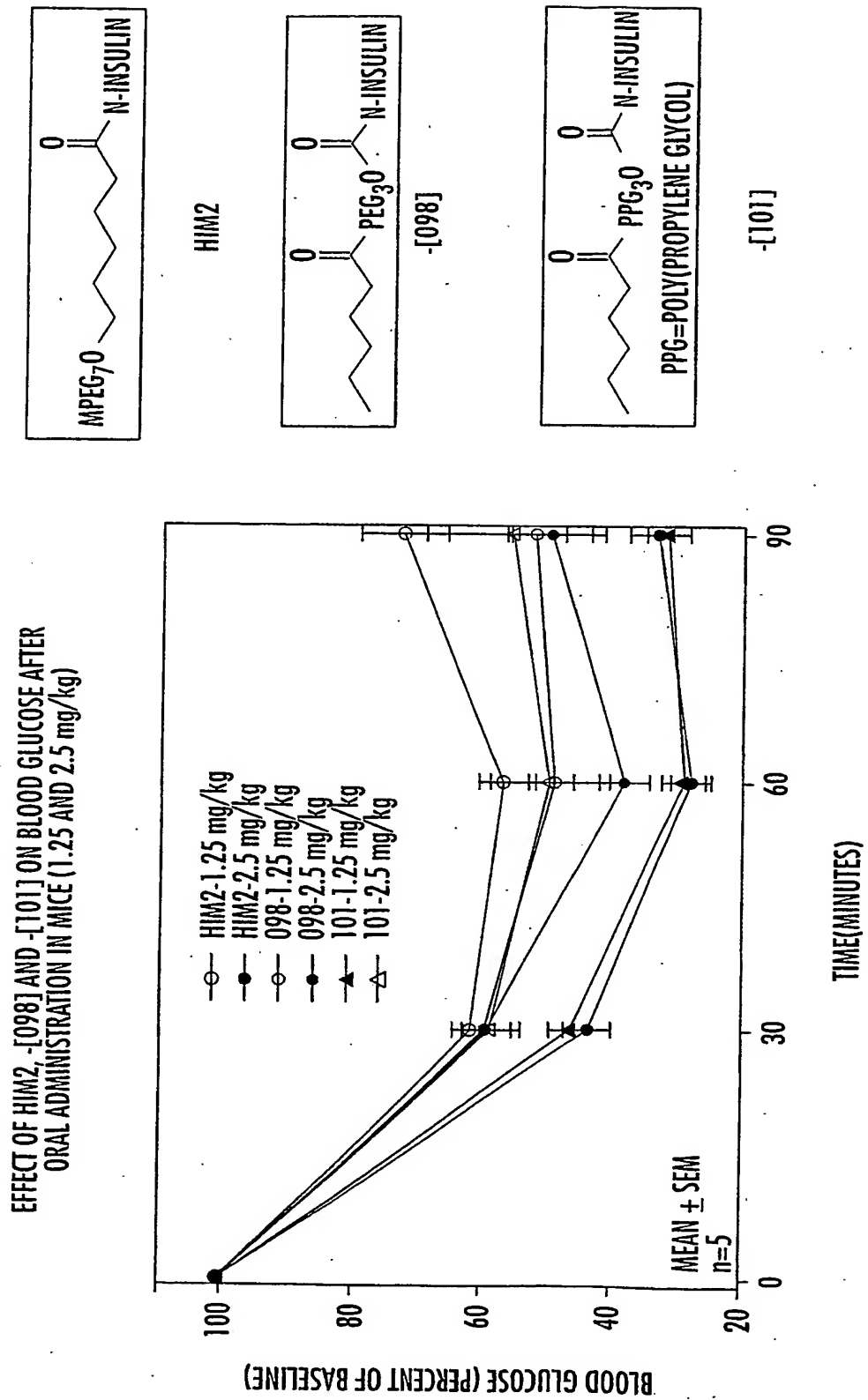


FIG. 9.

THIS PAGE BLANK (USPTO)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number
WO 03/022208 A3

- (51) International Patent Classification⁷: **A61K 38/28**, C07K 14/62 (74) Agent: MYERS BIGEL SIBLEY & SAJOVEC; PO Box 37428, Raleigh, NC 27627 (US).
- (21) International Application Number: PCT/US02/28429 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date:
6 September 2002 (06.09.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/318,193 7 September 2001 (07.09.2001) US
60/377,865 3 May 2002 (03.05.2002) US
- (71) Applicant (*for all designated States except US*): NOBEX CORPORATION [US/US]; Post Office Box 13940, Research Triangle Park, NC 27709-3940 (US).
- (72) Inventors; and
(75) Inventors/Applicants (*for US only*): SOLTERO, Richard [US/US]; 5509 Solomon's Seal Court, Holly Springs, NC 27540 (US). RADHAKRISHNAN, Balasingham [US/US]; 209 Faison Road, Chapel Hill, NC 27517 (US). EKWURIBE, Nnochiri, N. [NG/US]; 216 Coltsgate Dr., Cary, NC 27511 (US). REHLAENDER, Bruce [US/US]; 604 Highgrove Drive, Chapel Hill, NC 27516 (US). HICKEY, Anthony [GB/US]; 1208 Killington Court, Chapel Hill, NC 27517 (US). BOVET, Li, Li [US/US]; 110 Marin Drive, Chapel Hill, NC 27516 (US).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- (88) Date of publication of the international search report:
25 September 2003
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PHARMACEUTICAL COMPOSITIONS OF INSULIN DRUG-OLIGOMER CONJUGATES AND METHODS OF TREATING DISEASES THEREWITH

(57) Abstract: Pharmaceutical compositions that include an insulin drug-oligomer conjugate, a fatty acid component, and a bile salt component are described. The insulin drug is covalently coupled to an oligomeric moiety. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. Methods of treating an insulin deficiency in a subject in need of such treatment using such pharmaceutical compositions are also provided, as are methods of providing such pharmaceutical compositions.

WO 03/022208 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/28429

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/28; C07K 14/62

US CL : 514/3; 530/303

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/3, 4; 530/303, 304, 305

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,156,719 A (SEZAKI ET AL) 29 May 1979 (29/05/79), see entire document, especially the Abstract, Experiment 1.	51-53
---		-----
Y		1-50
X,P	US 2002/0018811 A1 (PENTEADO ET AL) 14 February 2002 (14/02/02), see entire document, especially paragraph 98.	51
A	JP 52057313 A (YAMANOUCHI PHARM CO LTD) 11 May 1977 (11/05/77), see entire document.	1-53
Y	WO 00/078302 A1 (PROTEIN DELIVERY, INC.) 28 December 2000 (28/12/00), see entire document, especially page 8, line 6 - page 11, line 26, page 38, line 1 - page 39, line 18.	1-50



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

02 October 2002 (02.10.2002)

Date of mailing of the international search report

14 MAR 2003

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Jeffrey E. Russel

Telephone No. (703)308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US02/28429

Continuation of B. FIELDS SEARCHED Item 3:

EAST, DIALOG

search terms: insulin, conjugate, fatty acid, oleic, capric, lauric, bile, cholic, cholate, deoxycholate

Form PCT/ISA/210 (second sheet) (July 1998)

THIS PAGE BLANK (USPTO)